ENDOCRINOLOGY

VOLUME 42 APRIL, 1948 NUMBER 4

THE USE OF THE MALE NORTH AMERICAN FROG (RANA PIPIENS) IN THE DIAGNOSIS OF PREGNANCY[1]

STANLEY L. ROBBINS[2] AND FREDERIC PARKER, JR.[3]

From the Mallory Institute of Pathology, Boston City Hospital

BOSTON, MASS.

WHILE it has been known for some time that the African clawed frog (Xenopus laevis) could be stimulated to mating by the injection of mammalian gonadotropins (Shapiro, 1936), this reactivity has only recently been utilized for the demonstration and titration of such hormones (Robbins, Parker and Bianco, 1947). It was found that the male frog reacted far more quickly than the female and furthermore was 64 times as sensitive to pituitary gonadotropin and 10 times as sensitive to chorionic gonadotropin.

These facts naturally led to an investigation of the possibility of the use of the male Xenopus laevis for the diagnosis of pregnancy. However, the animal proved unsatisfactory for such tests. The difficulties encountered and possible explanation of them will be discussed in another paper.

Ample evidence existed from previous work suggesting the possible use of other species of male frogs or toads. In 1929 Houssay, Giusti and Lascano-Gonzales (1929) demonstrated that the female South American toad (Bufo marinus) reacted, in the form of ovulation, to the implanation of toad pituitary. In this same year Wolf (1929) accomplished the same result in the female Rana pipiens, also using homologous pituitary. The effectiveness of mammalian pituitary in the female Rana pipiens was not demonstrated until many years later

Received for publication January 29, 1948.

[1] This work was performed under a research grant from Ciba Pharmaceutical Products, Inc.

[2] Assistant pathologist, Mallory Institute of Pathology; assistant professor of pathology, Boston University School of Medicine; instructor in pathology, Tufts College Medical School.

[3] Pathologist-in-chief, Mallory Institute of Pathology; associate professor of pathology, Harvard Medical School.

Copyright, 1948, by the Association for the Study of Internal Secretions

by Wright and Hisaw (1947). While these authors produced ovulation in the Rana pipiens, they called attention to the fact that other investigators, using essentially the same technic, had failed to achieve similar successful results. All this preceding work strongly suggested the probable universality of the reaction of frogs and toads to gonadotropic hormone of both amphibian and mammalian origin.

Based on this work a pregnancy test was recently described by Mainini using a male South American toad (Bufo arenarum Hensel) (1947). This toad, under the conditions of the investigation, appeared to have value in the routine diagnosis of pregnancy. Ninety-nine pregnancy tests were run in parallel with the Friedman test. In 94 instances the rabbit and toad agreed. In 5 tests "inconsistent" results were obtained, the toad being positive in 3 and the rabbit negative; in 2 others the rabbit was positive and the toad negative. Whether these "inconsistencies" could be construed to mean 3 false positive reactions and 2 false negative reactions was not clear. Seventy-seven control urines obtained from children, menopausal women and normal male and female adults, etc. were consistently negative. However, some doubt as to the practical usefulness of the test was raised by the author since, as he stated, " . . . it might happen that the reaction of the toad could be different during other seasons of the year, i.e. during the mating period or variations of tempature."

It is the purpose of this paper to make a preliminary report on the use and reliability of another species of male amphibian, the common North American frog (Rana pipiens) in the routine diagnosis of pregnancy.

The use of this frog has many significant advantages such as (1) abundant supply, it being indigenous to wide areas of the United States, (2) low cost, since the animals cost in the vicinity of 10 cents and 2–4 frogs suffice for individual tests, and (3) it reacts to untreated urine within 1–2 hours. Analyses on 112 urines from known pregnant women and women suspected of being pregnant form the basis of this report. In every instance where unequivocal clinical evidence of pregnancy, such as can be found in a 5–9 month gestation, was lacking, parallel tests were performed on rats and rabbits. An additional small series of urines from normal girls having regular menses was also tested.

TECHNIC

The technic of the performance of this test consists essentially in the subcutaneous administration of 5 cc. of urine to each of a pair of male frogs. The first morning urine is used as in other biologic tests for pregnancy. After simple filtration through ordinary filter paper to remove crystalline sediment and debris, 5 cc. of urine is injected subcutaneously into the dorsal lymph sac. No adjustment of pH or control of the temperature has been found necessary and the animals take this volume of fluid with great ease. Cloacal smears of the animal are then performed, beginning one hour later,

following the technic described in a previous report (Robbins, Parker and Bianco, 1947). It consists essentially of aspirating the fluid obtained from the external cloacal orifice with a glass capillary pipette. It is unnecessary to enter the cloacal cavity since ample fluid is always obtainable at the outlet. The cloacal fluid thus obtained is examined, as has been described, in a hanging drop preparation under reduced light. The spermatozoa are very readily seen, more easily than those of the Xenopus laevis, since they are considerably larger and the reactions are usually all or none, producing either large numbers of sperm (Figures 1 and 2) or none. It is important to note that the pres-

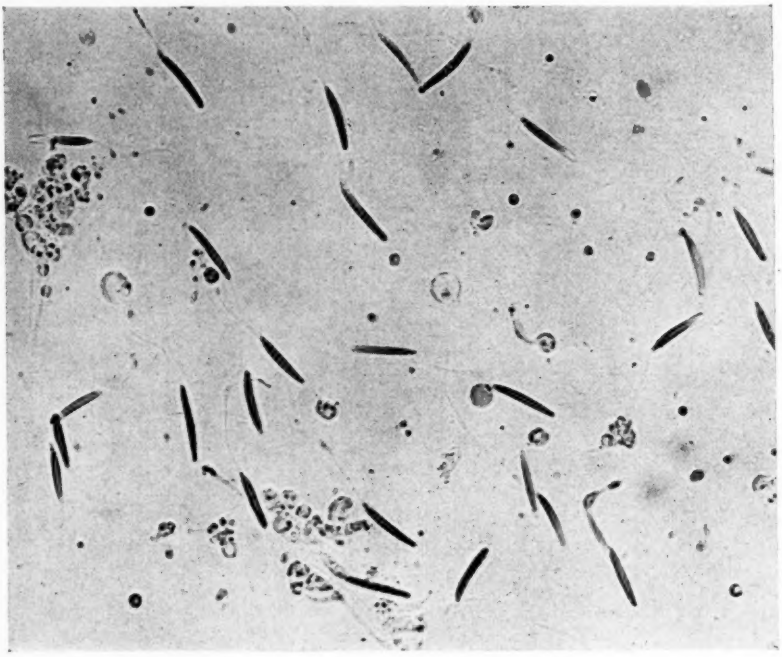


FIG. 1. The spermatozoa as seen in an unstained preparation under reduced light. ×800.

ence of any sperm, motile or not, is indicative of a positive reaction. When the first cloacal examination is negative it should be repeated in an hour. In the series of urines here reported positive reactions have never been delayed more than 2 hours. If a reaction does not develop within this 2-hour period after the administration of the urine, it is safe to conclude that the test is negative. The possibility of missing a positive reaction by the use of hourly examinations is quite remote. Once a reaction develops it persists at peak levels for 1–2 hours, and can be demonstrated at considerably lower levels for 4–5 hours.

Some of the urines tested were toxic, killing both frogs. Usually this toxicity is apparent within one-half hour after the administration of the urine, the frogs being either very sick or dead. In such cases the 5 cc. of urine is di-

vided into 2.5 cc. doses, given an hour apart. When this split dosage technic is employed, it is advisable to make an examination of the cloacal fluid before administering the second injection. In the present series most of the positive urines tested with the split dosage reacted after the first injection. Hence, not only was the second injection unnecessary, but moreover considerable time was saved in obtaining the positive result. This splitting of the 5 cc. volume of urine appears to diminish the toxicity of the test, while not appreciably affecting the sensitivity.

At the termination of the test it has been found most convenient to simply discard the frogs that have been used without attempting to re-use them.

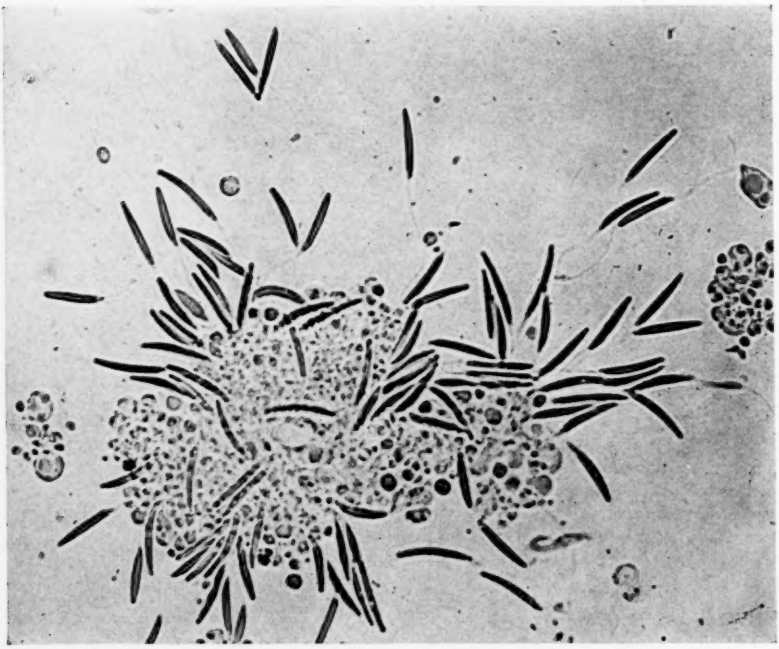


FIG. 2. Typical aggregations of spermatozoa found in most strongly positive reactions, unstained. ×800.

RESULTS

To date a total of 122 urines have been tested. Ten of these urines were obtained from normal girls having normal menses. The remaining 112 urines comprised 78 known pregnancy urines, the other 34 urines being obtained from women with amenorrhea determined not to be pregnant by the Aschheim-Zondek test or its Friedman modification. The results are charted in Table 1.

As the table indicates, no false positive reactions have ever been encountered. In one instance a false negative test was obtained. The false negative was encountered in a urine from a woman 2 weeks after

TABLE 1. RESULTS OF FROG TESTS

Source of urine	Correct positive	Correct negative	False positive	False negative
Pregnancy	77			1
Amenorrhea without pregnancy		34		
Normal menstruating women		10		
	77	44	0	1

her first missed period, or at most 3–4 weeks pregnant. The rabbits and rats run in parallel on this urine gave extremely weak reactions, of the type considered to be minimal for the diagnosis of pregnancy in this laboratory. While the above test gave a false negative result, urines from two other patients with an assumed duration of gestation of 3–4 weeks gave a strong positive reaction, presumably having a somewhat higher titer.

DISCUSSION

In any discussion of a new pregnancy test the most important question is what is the reliability of the test? As can be seen from the above results in the series of 122 urines examined, one false negative test has been encountered. More significant, however, is that there have been no false positive tests. The one false negative test merits comment.

The false negative test was encountered in a urine from a woman presumably 2 weeks after her first missed period, undoubtedly at a time when the gonadotropic titer was still very low. Since two other women at the same presumed stage of gestation gave strong positive results, it can only be assumed that the urine giving the false negative result was of unusually low titer for this stage of pregnancy. To date, the limits of the sensitivity of this test have been little explored. However, in the present series of urines tested, the earliest pregnancy detected was that of a patient 7 days after her first missed period. A comparison by serial dilutions of a single known positive urine on a series of male Rana pipiens and immature female rats showed the frog and immature rat to be approximately equally sensitive. It would appear from these solitary observations that the sensitivity of this male frog compares favorably with the immature female rat.

The problem of the false negative reaction, while by no means serious, bears further investigation. The possibility of using larger volumes of whole urine is complicated by the high toxicity of these larger doses for the frog. However, since the frogs are so cheap and easy to obtain and where some evidence exists for the possibility of the rare false negative reaction, little can be lost by the use of larger volumes of urine.

Occasionally urines are toxic to the frogs in even 2.5 cc. amounts. As in the Aschheim-Zondek test and Friedman test, it is customary under these circumstances to repeat the test on a new sample of

urine. Methods of detoxification of urines are sorely needed for all bioassay technics. Work on this problem is being continued.

As can be seen from the technic described, it has been customary to not re-use the frogs. This fact does not imply that re-use of the animals is impossible or not feasible, since on rare occasions individual frogs that have been used on negative tests have been successfully used over again with completely satisfactory results. However, since these amphibia are difficult to feed, living chiefly on live insects, and since under laboratory conditions their metabolic processes are stepped up over hibernating levels, it has been considered wisest to not permit inanition or wasting to develop by prolonged use of these animals. The one occasion when frogs were used that had been kept under usual laboratory conditions for several months without attempts at feeding proved beyond a doubt that such animals were completely unsatisfactory for use, since they failed to react to urines that gave positive results in frogs not subjected to long periods of laboratory storage. The fact that these amphibia inhabit almost the entire United States, save for a narrow zone along the Pacific coast, makes them readily available in this country.

Sufficient time has not elapsed to permit study of the problem of the use of these animals throughout the year. The Rana pipiens used in this investigation, carried on in the winter months, were obtained from Vermont where they had been kept in artificial hibernation at the bottom of a lake. Since hibernation is presumably the period of lowest metabolic activity and re-activity, it seems reasonable to assume that they should prove utilizable throughout the year. What precautions, if any, may have to be taken during their breeding season are as yet not known.

SUMMARY

A new test for pregnancy, utilizing the release of spermatozoa by the male Rana pipiens on stimulation with mammalian chorionic gonadotropin is described.

Its advantages are many. Small amounts (5 cc.) of whole urine can be administered directly to each frog, making the test very simple. Reactions to positive urines develop sometimes within a half-hour and invariably within 2 hours, producing a test considerably faster than any other previously described biologic test. The animals are widely available throughout the continental United States and as a result are extremely cheap; thus the cost of the test is most reasonable.

In a carefully controlled series of 122 urines no false positive reactions were encountered. One false negative reaction occurred on a urine early in the period of gestation.

The ease with which a diagnosis of pregnancy can be established with this test makes possible the adoption of this technic in any laboratory, however small. The rapidity that it affords makes of the test

an emergency procedure of possible benefit in difficult diagnostic problems.

ACKNOWLEDGMENTS

The authors wish to express their grateful appreciation to Miss Phyllis D. Bianco and Miss Gloria E. Mannix for their unstinting technical assistance.

ADDENDUM

Since this paper was submitted for publication P. B. Wiltberger and D. F. Miller (*Science*, Vol. 107, p. 198, 1948) described the use of Rana pipiens as a test for pregnancy and found it to be of high diagnostic value.

REFERENCES

HOUSSAY, B. A., L. GIUSTI AND J. M. LASCANO-GONZALES: *Rev. Soc. argent. de biol.* 5: 397. 1929.
MAININI, C. G.: *J. Clin. Endocrinology* 7: 653. 1947.
ROBBINS, S. L., F. PARKER, JR. AND P. D. BIANCO: *Endocrinology* 40: 227. 1947.
SHAPIRO, H. A.: *J. Exper. Biol.* 13: 48. 1936.
WOLF, O. M.: *Proc. Soc. Exper. Biol. & Med.* 26: 692. 1929.
WRIGHT, P. A., AND F. L. HISAW: *Endocrinology* 39: 247. 1946.

EXPERIMENTAL DIABETES PRODUCED BY THE ADMINISTRATION OF GLUCOSE

F. C. DOHAN AND F. D. W. LUKENS
From the George S. Cox Medical Research Institute, University of Pennsylvania
PHILADELPHIA, PENNSYLVANIA

A PERSISTENT INCREASE in the concentration of blood glucose has frequently been considered a factor in the production of damage to the islands of Langerhans. Some of the experimental data suggesting that this may be so is as follows: (a) The experiments of Allen (1922) and Homans (1914) indicate that in subtotally depancreatized dogs or cats a period of hyperglycemia precedes the occurrence of hydropic degeneration of the beta cells. (b) In dogs (Dohan, Fish and Lukens, 1941) or partially depancreatized cats (Lukens and Dohan, 1942) injected with crude anterior pituitary extracts hyperglycemia precedes the hydropic degeneration of the beta cells. (c) The hydropic degeneration produced by anterior pituitary extract may be prevented or reversed by means of diet, insulin or phlorizin (Haist, Campbell and Best, 1940; Lukens and Dohan, 1942; Lukens, Dohan and Wolcott, 1943). All these measures tend to maintain a normal concentration of blood glucose.

These and other considerations led us to attempt the production of hydropic degeneration and permanent diabetes by maintaining an increased blood glucose concentration by glucose injections. Previous experiments (Jacobs and Colwell, 1936; Astwood, Flynn and Krayer, 1942; Dohan and Stengel, unpublished) using the intravenous route suggested that intraperitoneal injections might be more satisfactory. Cats were found to tolerate such injections better than dogs, rabbits or rats. Part of this work has been reported in preliminary form (Dohan and Lukens, 1947). Previous efforts to influence the islands of Langerhans by the administration of glucose will be cited in discussing the results.

METHODS

Animals. Thirty-five apparently healthy cats of both sexes were used. Eight of these animals had approximately the splenic half of the pancreas removed under pentobarbital anesthesia. After several weeks, when preoperative weight, appetite and well being were completely restored they were considered suitable for experimental purposes. None of the operated cats developed spontaneous glycosuria.

Received for publication February 4, 1948.

Injections. The standard solution for injection contained 20% glucose and 0.9% sodium chloride. This was boiled for 5 to 10 minutes after preparation and kept in flasks in the ice box. After cooling, 100 mg. of thiamine hydrochloride was added to each liter of glucose solution. This solution was warmed to body temperature before injection and all injections were made intraperitoneally through the shaved and washed abdominal wall. Injections were usually made about 8:30 A.M., 4:30 P.M., and 10:30 P.M. and the dose varied from 50 cc. to as high as 420 cc. in 24 hours. Smaller and less frequent doses were used in the first few days. The clinical conditions of the animal, distention of the abdomen, glycosuria and glycemia were used as indications of the amount and frequency of injections.

Diets. In general the cats were fed measured diets of ground meat with a few grams of powdered yeast, cod liver oil and bone ash added. At times the supplements were omitted or canned salmon was offered as an inducement to eat. Usually the appetite was greatly reduced during the period of injection.

Chemical. The cats were kept in metabolic cages and urines were collected in the usual manner. Blood samples for glucose determination (Somogyi, 1945) were taken from the ear. The majority of such observations were made on samples taken just prior to the intraperitoneal injections and so represented the lowest values in the 24 hour period. For these values the term *pre-injection* blood glucose will be used. In some animals *pre-injection* blood glucose was determined before every injection, in others once or twice daily to ascertain the response and adjust the dose of glucose.

The following analytical methods were employed: urinary glucose (Benedict, 1911), cholesterol (Bloor, 1916), fatty acid (Stoddard and Drury, 1929), glycogen (Cori, 1932), absorbic acid (Roe and Kuether, 1943), galactose (Raymond and Blanco, 1928).

Histological. At autopsy the tissues removed for histological examination were fixed in 10% formalin. The pituitary and an additional section of the pancreas were placed in Zenkers bichromate solution without acetic acid. The Zenker fixed tissues were washed at 8–12 hours and the pancreas was stained by the Bensley-Mallory method; the pituitary by Mallory's method. Hematoxylin and eosin stains were used for the other tissues.

RESULTS

The course of hyperglycemia and the production of hydropic degeneration of the islets during glucose administration

Thirty-five cats were given 1 to 3 intraperitoneal injections of 20% glucose in 0.9% sodium chloride solution per day. Of these, 10 developed hydropic degeneration of the islands of Langerhans, and of this group 3 exhibited persistent diabetes after the cessation of glucose administration. Twenty-five cats failed to develop hydropic degeneration of the islands of Langerhans. Many of the animals, as will be discussed later, developed a syndrome of anorexia, ataxia, weakness and death. The results will be illustrated by the course of events in a single cat.

Figure 1 illustrates the food intake, the glucose injected and excreted and the daily minimal (i.e., *pre-injection*) blood glucose concen-

trations in a normal female cat (G-13) injected for 27 days. It will be noted that the amount of glucose injected was gradually increased from 10 grams the first day to 72 grams per day for the last 4 days before autopsy. After the second day injections were given three times a day at approximately 8, 6 and 10 hour intervals. After the fourth day of injection the majority of values for blood glucose were above 200 mgm. per 100 ml. During the last half of the period there was an increased proportion of injected glucose excreted in the urine.

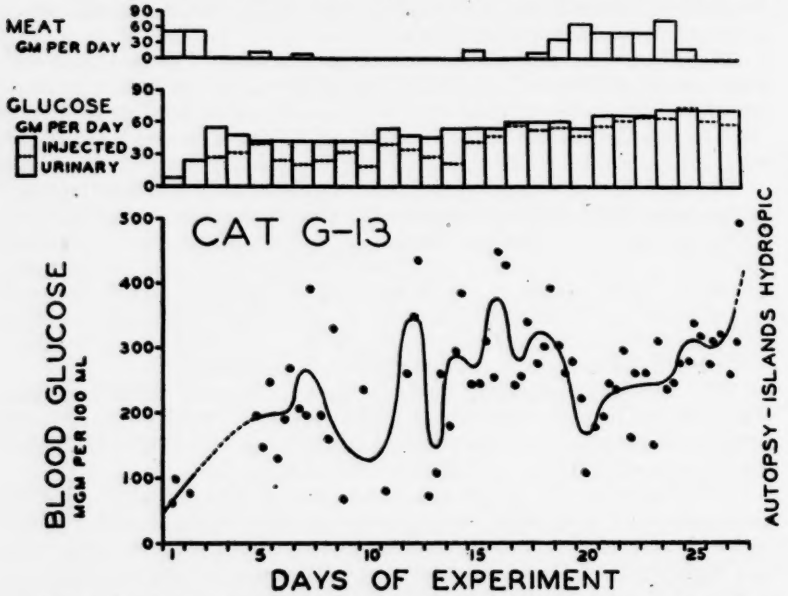
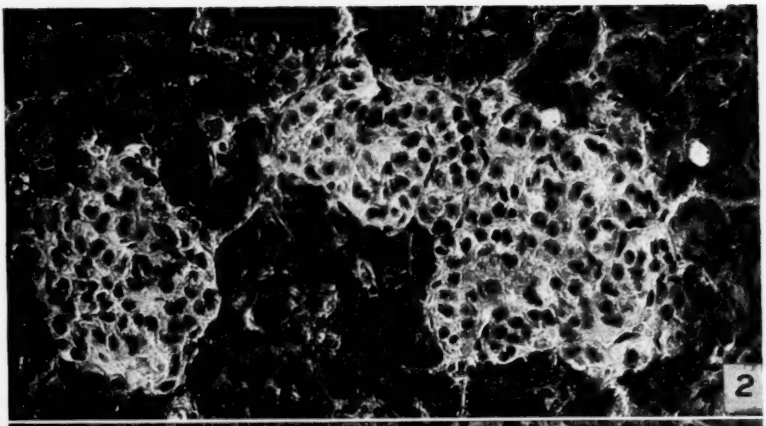
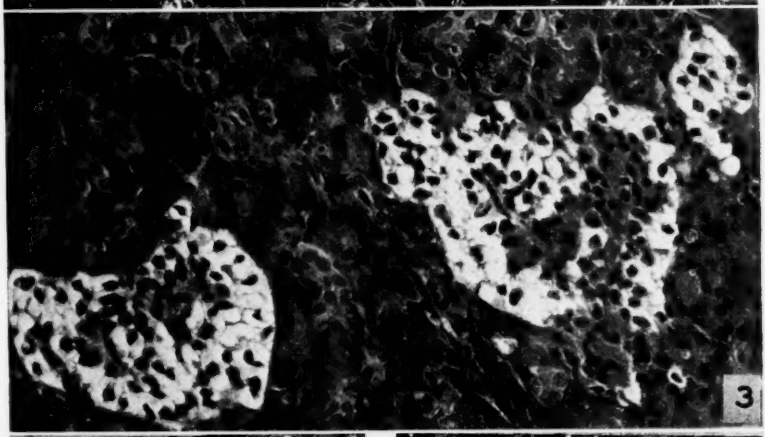
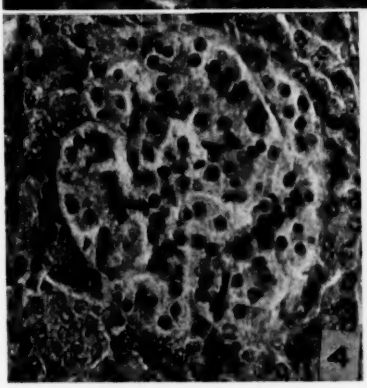


FIG. 1. The Production of Hyperglycemia and Islet Damage by the Administration of Glucose. This normal female cat was injected with 20% glucose in 0.9% saline solution for 27 days. Injections were given 3 times a day after the second day. Black dots indicate the blood glucose concentration just before each injection of glucose. The curved line indicates the averages of the daily observations. The hydropic degeneration of the islands of Langerhans is illustrated in Fig. 3. See also Tables 1 and 2.

For the 3 days prior to autopsy the cat excreted 89% of the total available glucose. In addition to the thiamine hydrochloride added to the injection fluid, yeast and cod liver oil were offered in the ground meat both before and during the period of glucose injections. As was true with the majority of cats, little food was eaten during the injection period. By the end of the 27th day the animal had lost 23% of its original body weight and was quite weak. Pentobarbital was given intraperitoneally and an autopsy was performed. The terminal blood glucose concentration (uncharted) was 1040 mgm./100 ml. 9 hours after the last injection of glucose. No evidence of peritonitis or other gross lesion was found. Sections from the pancreas showed marked







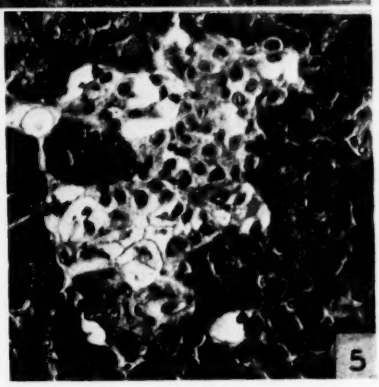


FIG. 2. Islands of Langerhans of normal cat. ×330.

FIG. 3. Islands of cat G-13 showing hydropic degeneration of beta cells. By Mallory stain the uninjured cells were alpha cells. ×330. See Fig. 1.

FIG. 4. Island of cat G-25. Slight degranulation of the cells when compared to the normal Fig. 2. ×330. See Fig. 7.

FIG. 5. Island of cat G-43. Irregular shape of island and small number of hydropic beta cells indicate beginning atrophy. ×300.

hydropic degeneration of the islands of Langerhans (Fig. 3). Sections of the liver, kidney, pituitary, thyroid, adrenal and ovary revealed no obvious abnormalities.

Relation of islet damage to the number of days of hyperglycemia

Table 1 shows the *days of hyperglycemia*, i.e., the number of days on which the morning blood glucose was 149 mgm. per 100 ml. or

TABLE 1. ISLET LESIONS RELATED TO DURATION OF HYPERGLYCEMIA

Cat No.	Days of hyperglycemia[1]	Hydropic degeneration of beta cells	Weight of pancreas	Period of injection	Average[2] *pre-injection* blood-glucose
	days		gm.	days	mg./100 ml.
10 Cats Exhibiting Hydropic Degeneration of the Beta Cells					
5 Normal Cats					
G12	9	+	—	14	326
G13	19	++++	—	27	250
G19	20	++++	—	29	251
G16	33[3]	++++	5.0	38	216
G20	44	++++	10.7	104	198
Average	25		—	42	248
5 Partially Depancreatized Cats					
G 3	13	+++	2.05	30	209
G 8	14	++++	1.90	15	282
G46	29	+++	4.06	50	222
G43	31[3]	++++	5.50	52	270
G51	38[3]	++++	4.60	55	277
Average	25		3.62	40	252
25 Cats not Exhibiting Hydropic Degeneration of Beta Cells[4]					
Average	2	None	6.3[5]	9	115
Range	0–8	—	3.5–11.1	3–21	64–553

[1] Number of days on which the blood glucose concentration was greater than 149 mg./100 ml. immediately before the morning injection of glucose.
[2] Average of all blood sugars taken just before the intraperitoneal injections of glucose during the period of injection.
[3] Diabetes persisted after glucose administration was stopped.
[4] 22 Normal and 3 partially pancreatectomized cats.
[5] Pancreatic weight in 18 normal cats injected with glucose.

higher. These days were usually but not always consecutive. The period of hyperglycemia thus estimated is related to the status of the pancreatic islets. The morning blood glucose value was chosen as an index of the duration of hyperglycemia because the glucose concentration was determined more often at that time. Furthermore, in the great majority of instances it also proved to be the lowest of the 3 daily *pre-injection* values presumably because of the longer interval after an injection. It will be noted that the 10 cats which showed hydropic degeneration of the islets, had morning blood glucose values of 149 mgm./100 ml. or greater on 9 or more days, whereas the cats without lesions had "hyperglycemia" on only 8 days or less. The dura-

tion of hyperglycemia is also related to the total period of injection, to the average blood glucose of this experimental period and to the pancreatic weight. The effect of duration of the elevation of blood sugar is further illustrated in Figure 6, which relates the average of all *pre-injection* blood glucose values and the duration of injections to the islet pathology of the normal and partially depancreatized cats. The range of average blood glucose values for the cats with lesions was *198* to *326* mgm./100 ml. while for the cats without lesions

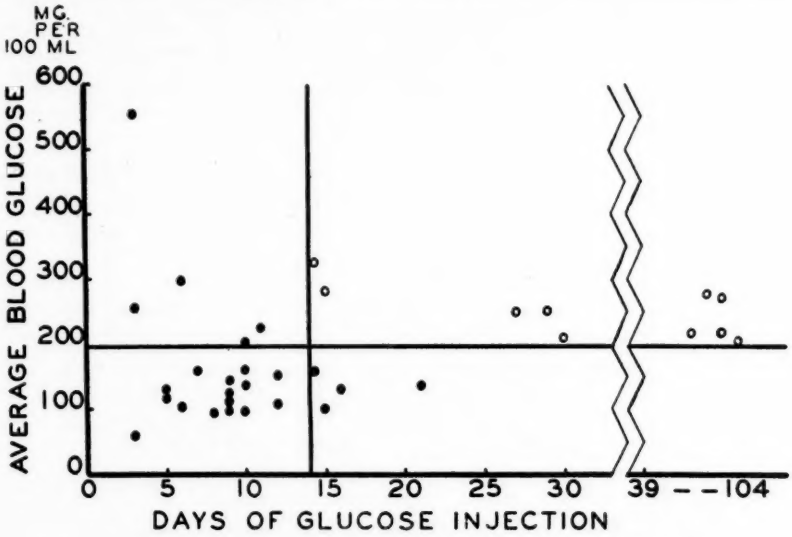


FIG. 6. Islet Lesions, *Pre-Injection* Blood Glucose Concentrations and Duration of Injections.
○ = Cats with hydropic degeneration.
● = Cats without hydropic degeneration.

it was *64* to *553* mgm./100 ml. Particularly striking is the fact that 2 partially depancreatized and 3 normal cats exhibited average blood glucose levels of 196 mgm. or higher during the injection period of 11 days or less yet failed to show degeneration of the islets. For emphasis the chart has been divided into 4 quadrants. The cats without lesions but with high average *pre-injection* blood glucose concentrations are grouped in the left upper quadrant, and the 10 cats with lesions in the right upper quadrant. The symbols in the right lower quadrant show that those cats not exhibiting high average blood glucose concentrations even though injected for weeks, did not develop hydropic degeneration of the islands.

An example of a cat having high blood glucose concentrations for less than 9 days is presented in Figure 7. From the 3rd day to the 10th day inclusive this cat exhibited a moderate pre-injection hyperglycemia

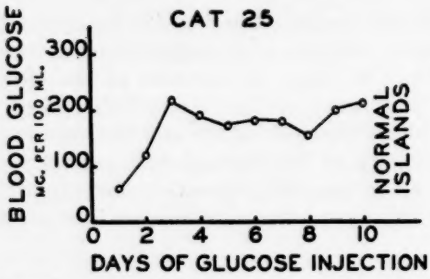


FIG. 7. Hyperglycemia of Short Duration without Hydropic Degeneration of the Islets. Normal female cat injected intraperitoneally 3 times daily with 20% glucose solution for 10 days. Initial weight 2.90 kg. Final weight 2.80 kg. Average daily dose of glucose was 14.3 gm. per kilogram of initial body weight. Circles indicate *pre-injection* blood glucose concentration. See Fig. 4.

yet failed to show hydropic degeneration of the islets as did those animals having hyperglycemia. A photomicrograph of the pancreas is shown in Figure 4.

Metabolism during the period of glucose injections

Some of the metabolic data collected during the period of glucose injections is tabulated in Table 2. As in Table 1, the cats with lesions

TABLE 2. METABOLISM DURING GLUCOSE INJECTIONS

Cat[1] No.	Period of injection	Average glucose injected	Average glucose Excreted	Available[2] glucose Excreted last 3 days	Average[2] caloric intake	Average[2] calories utilized	Weight Initial	Weight Final
	days	gm./k/d	gm./k/d	per cent	cal./k/d	cal./k/d	kg.	kg.
10 Cats Exhibiting Hydropic Degeneration of the Beta Cells								
5 Normal Cats								
G12	14	16.2	8.2	66	71	38	2.78	2.78
G13	27	18.9	14.6	89	80	32	2.78	2.15
G19	29	17.3	10.5	76	109	67	2.15	1.65
G16	38	11.4	10.0	87	100	60	3.38	2.75
G20	104	13.9	11.9	95	133	85	3.20	3.05
Average	42	15.5	11.0	83	99	56	2.86	2.48
5 Partially Depancreatized Cats								
G 3	30	8.5	6.6	107	34	8	3.98	2.65
G 8	15	5.8	4.8	95	23	4	4.82	3.50
G46	50	9.6	7.0	94	69	41	3.40	2.23
G43	52	9.4	9.0	89	96	60	3.15	2.75
G51	55	16.2	10.0	93	133	93	3.20	2.48
Average	40	9.9	7.5	96	71	41	3.71	2.72
25 Cats Not Exhibiting Hydropic Degeneration of Beta Cells								
Average	9	11.2	3.2	31	71	58	3.74	3.27
Range	3–21	6.2–22.7	0.4–8.8	0–75	25–129	13–124	2.1–4.8	2.2–4.8

[1] Tabulated in same order and grouped as in Table I.
[2] Gross approximation using standard tables and methods of calculation.

are listed according to the number of days upon which a morning hyperglycemia of greater than 149 mgm. % was present. The findings in the normal and partially depancreatized cats with hydropic degeneration are listed individually and may be compared with the average and the range of the findings in those cats without hydropic degeneration. *The average amount of glucose injected* varied considerably but, as expected, the amount necessary to keep the partially depancreatized cats hyperglycemic was less than that used in the normal cats. The wide range and lower average amount of glucose injected into the cats without lesions is a reflection of the fact that this group included most of those that became weak and ataxic as a result of the injections (see below) and, because of this, injections were often decreased or stopped.

As another indication of the severity of the diabetes the proportion of the available glucose excreted in the urine in the 3 days preceding autopsy is shown in the fifth column. It will be noted that the animals

TABLE 3. THE RESPONSE OF BLOOD SUGAR TO SINGLE GLUCOSE INJECTIONS

	Blood glucose		
	Pre-injection	4 hours	8 hours
	mg./100 ml.	mg./100 ml.	mg./100 ml.
Average	113 (40)*	347 (36)	236 (27)
Range	55–262	158–510	63–448
Average 5 highest	210	446	310
Average 5 lowest	58	286	137

* Figures in parentheses indicate number of observations.

with lesions excreted a high proportion of available glucose, ranging from 66% to 107%. The lowest amount excreted was found in cat G-12 which exhibited the least hydropic degeneration of the pancreatic islets (see Table 1).

A gross approximation of the *total caloric intake* has been made from the food ingested and the glucose injected. It will be noted that the caloric intake in general was the same or less than that usually ingested by healthy cats, i.e., the equivalent of 150–200 grams of meat per day for a cat weighing 3 kilograms. *Body weight* was lost in all but one of the cats with lesions and in most of the cats without lesions. This was due to glycosuria and decreased food intake. During the period of injections large amounts of intraperitoneal fluid were frequently present. At autopsy the animals were weighed before opening the abdomen so that the "final weight" was distorted by this gross disturbance of fluid balance.

The effect of intraperitoneal injections of glucose upon the concentration of blood glucose during the subsequent hours has been measured. In general, blood samples were obtained just before the injection and at the 4th and the 8th hour thereafter although a few complete hourly curves were made. The great majority of observations were made during the first to the 14th day of injections. Great

variability in effect was noted as was expected from the variation in the amount of glucose injected, the amount of fluid in the peritoneal cavity (resulting from the previous injections), the *pre-injection* blood glucose levels and general condition of the animals. The summary in Table 3 represents observations on 40 days in 20 cats. The 4th hour blood glucose value in particular indicates the transitory high values which the blood glucose concentration may attain.

The production of persistent diabetes

A persistent hyperglycemia and glycosuria has followed the period of intraperitoneal glucose injections in 1 normal cat and 2 cats with half of the pancreas removed. The course of events in the normal cat (G-16) is shown in Figure 8. This cat was injected 3 times a

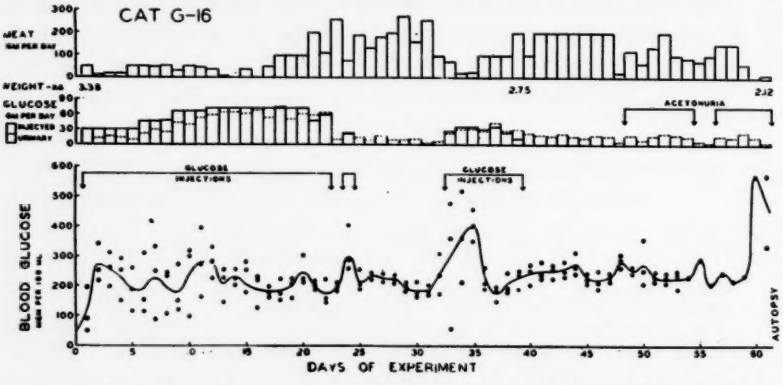


FIG. 8. Producton of Permanent Diabetes by Gluclose Injections. This normal male, cat was given 3 intraperitoneal glucose injections per day with 2 intermissions, over a period of 39 days. The black dots indicate the *pre-injection* blood glucose values during the period of injection and later the blood glucose at similar times when glucose was not being injected. The curved black line indicates the average of the daily observations.

day for a period of 39 days. A trial without injections was made from the 25th day to the 32nd day inclusive. Injections were then resumed on the 33rd day because of a blood sugar value of 58 mgm./100 ml. found that morning. The last injection of glucose was given on the 39th day. It will be noted that throughout most of the injection period a high concentration of glucose was present in blood samples taken just before each injection. These represent the lowest blood sugar values during the day. After stopping injections on the 39th day the cat was observed for 22 days during which it lost 0.67 kilograms of weight and the hyperglycemia and severe glycosuria continued. Slightly more than the calculated available carbohydrate of the meat diet was excreted during this period. On the 10th day after injections were stopped acetonuria was noted. By the 22nd day after the last injection (Fig. 8, day 61) the animal was obviously weak and de-

pressed and showed clinical evidence of acidosis. Pentobarbital was given intraperitoneally and autopsy was performed. The liver contained 1.04% glycogen and 21.2% of fatty acids, values similar to those obtained in untreated depancreatized cats. The blood obtained at time of autopsy also showed values to be expected in uncontrolled severe diabetes; thus the serum had a milky appearance and contained 1.60% of fatty acids, cholesterol was 243 mgm./100 ml., and urea nitrogen 105 mgm./100 ml. and the serum carbon dioxide content was 11 vols. per cent. Inspection revealed no evidence of infection and aside from the loss of subcutaneous fat and grossly fatty liver no gross lesions were noted. The liver weighed 95 grams, the pancreas 5.0 grams (normal) and the adrenals 710 mg. (above normal). Histological examination revealed severe hydropic degeneration of the islands of Langerhans. The liver was infiltrated with fat and other organs showed no noteworthy abnormality.

Two other cats, with half the pancreas removed, developed persistent post-injection diabetes. Data concerning these two cats, G-51 and G-43, may be found in Tables 1 and 2 which are supplemented by the following details. Cat G-51 weighed 2.48 kg. at the time glucose injections were terminated on the 55th day. After this it survived 8 days during which the average glycosuria was 77% of the available glucose of the meat diet. Anorexia and weight loss to 1.95 kg. preceded its death on the 9th post injection day and autopsy failed to reveal the cause of death. The liver fatty acids were normal (2.2%) and all tissues examined were microscopically normal except for the pancreas which showed marked hydropic degeneration of the islands of Langerhans. In the third cat (G-43) after 52 days of glucose injections severe diabetes persisted for the next 50 days. From the 50th to the 82nd day after glucose the animal continued to have glycosuria but in decreasing amounts. From the 83rd to the 97th day after glucose injection there was no glycosuria and normal blood sugar values were found. Despite the disappearance of diabetes there were marked changes in the pancreatic islands. They were decreased in number and the majority were very small with only a few hydropic beta cells remaining. Figure 5 illustrates one of the largest islands found. This animal which, during and after injections, had a total period of glycosuria of 6 months had atrophy of the islands resembling that seen late in pituitary-diabetes. The terminal disappearance of glycosuria is unexplained. (Although not found in this cat in the few sections examined, an adenoma of the type found in 3 other animals of this series might explain the results.)

The harmful effects of prolonged intraperitoneal administration of large amounts of glucose

A syndrome of anorexia, weakness of the hind legs and ataxia, progressing to severe weakness and death in animals injected intra-

venously, subcutaneously or intraperitoneally with large amounts of glucose for several days or more has been noted previously (Allen, 1913; Jacobs and Colwell, 1936; Astwood, Flynn and Krayer, 1942). These phenomena have appeared in all but a few of our animals without lesions. It was for this reason that larger amounts of glucose were not injected in these animals. It also occurred in a few of the cats showing hydropic degeneration of the islets. As anorexia was the rule during injection, early weakness was determined before each injection by the unsteadiness of the cat when dropped a few feet onto the

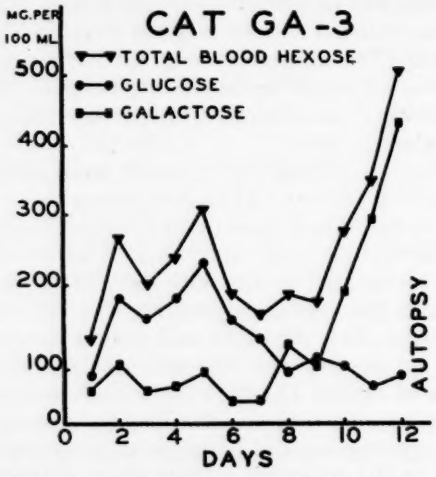


FIG. 9. This partially depancreatized cat without diabetes was injected with 20% galactose in 0.9% saline solution for 11 days. At autopsy on the 12th day the islets showed slight hydropic degeneration.

floor. However when weakness appeared it was usually progressive despite the cessation of injections and the use of the measures indicated below. Slight weakness of the hind legs might be followed in 12 to 24 hours by generalized weakness and death. Occasionally generalized convulsions appeared. Because of some similarity of the syndrome to thiamine deficiency large amounts of thiamine and cocarboxylase[1] were given at the first sign of weakness. These substances as well as riboflavin, niacin, pyridoxine, physiological saline, potassium chloride, sodium bicarbonate and adrenal cortical extract were given to various animals without conclusive evidence of beneficial effect. The pathogenesis of this syndrome remains obscure. We have also observed it during prolonged intraperitoneal injections of galactose. In this connection, Figure 9 suggests the failure to convert galactose to glucose in the 3 days before the syndrome developed. "Hepatic failure" of some kind may be the underlying cause of these

[1] Kindly supplied by Dr. Randolph T. Major, Merck and Co., Rahway, N. J.

fatalities from glucose injection, although the liver which was regularly sectioned at autopsy showed no lesions. The central nervous system was not examined.

Galactose injections. To determine if hydropic degeneration of the islets of Langerhans is a specific response to the increased concentration of blood glucose a 20% solution of galactose in 0.9% sodium chloride solution was injected intraperitoneally in two cats. One normal cat was injected for 20 days with doses up to 72 grams per day but failed to develop *pre-injection* hyperhexosemia and at autopsy there was no hydropic degeneration of the islets. The other cat injected with galactose had had two thirds of its pancreas removed. 104 days before the first injection. During the period of injection frequent determinations of blood glucose and galactose concentrations were made. Figure 9 shows the results. During the first part of the experiment the concentrations of blood glucose were considerably greater than those of galactose but during the latter part of the 12 day period the reverse was true. The animal was sacrificed early on the 12th day as it had suddenly become weak and ataxic, exhibiting the same clinical picture shown by many of the cats which died during the injections of glucose. Autopsy showed the pancreatic remnant to weigh 2.16 gm. and the islets showed slight hydropic degeneration. It is clear that the conversion of galactose to glucose is so efficient that this method fails to solve the problem of the specificity of glucose as a cause of island lesions.

The response of the adrenal glands to the intraperitoneal injection of glucose

In response to a variety of stimuli there is an increased secretion of adrenotrophic hormone and hence of adrenal cortical hormone (Long, 1947). The measurement of this response by the fall in adrenal ascorbic acid and cholesterol, as described by Sayers, Sayers, Liang and Long (1946) and Sayers and Sayers (1947), has been employed. In Table 4 the data on rats are in fair agreement with the results obtained by Sayers and Sayers (1947) after other stimuli such as cold

TABLE 4. ADRENAL ASCORBIC ACID AND CHOLESTEROL AFTER INTRAPERITONEAL GLUCOSE

Conditions	No. of animals	Adrenal wt. mg./100 gm. body wt.	Ascorbic Acid		Cholesterol	
			mg./100 gm. fresh adrenal tissue	micrograms per 100 gm. body wt.	mg./100 gm. fresh adrenal tissue	mg./100 gm. body wt.
Normal rats	7	20.6±0.5*	388±4.5	79.0±1.0		
Rats; 35–60 mins. after glucose	4	16.6±0.3	295±8.0	48.8±1.9		
Normal cats	5	13.6±0.5	102±2.1	14.1±0.5		
Cats; 1½ hrs. after glucose	14	16.2±0.2	88±1.3	13.9±0.2		
Normal cats	7	16.4±0.8			4.34±0.12	0.66±0.02
Cats; 1½ hrs. glucose	6	17.3±0.8			2.96±0.24	0.51±0.04

* Mean and standard error.

and adrenotrophic hormone. The 40% fall in adrenal ascorbic acid is significant. In cats, the concentration of ascorbic acid in the adrenals fell significantly but when the adrenal ascorbic acid per 100 gm. body weight was examined the fall was not statistically significant, because of the low adrenal weight in the control series. The decrease in adrenal cholesterol was significant for both units of measurement. In addition to these measurements, a few lymphocyte counts have shown consistent lymphocytopenia after the intraperitoneal injection of glucose. This lymphocytopenia conforms to the response to injury described by Selye (1946), Dougherty and White (1944) and by Freeman and Elmadjian (1946), the last authors having observed

TABLE 5. ADRENAL WEIGHTS OF CATS UNDER VARIOUS CONDITIONS

Conditions	No. of cats	Average body weight	Average adrenal weight	Adrenal weight / Body weight
		kg.	gm.	gm. per kg.
Normal	15	3.43 ± .18	0.586 ± .037	0.174 ± .010
Glucose Injections 5 to 105 days	11	3.34 ± .30	0.620 ± .056	0.183 ± .011
Anterior Pituitary Extract 6 to 11 days	6	2.96 ± .40	0.775 ± .135	0.250 ± .028
Hypophysectomized 11 to 85 days	6	3.19 ± .18	0.252 ± .023	0.080 ± .004

this response during glucose tolerance tests in man. All criteria considered, it was apparent that cats varied considerably in their reactions to intraperitoneal glucose. In other words, the injection of glucose appeared to be a weaker and less consistent stimulus of the adrenals than the carefully regulated procedures of Sayers and Sayers (1947). Similar chemical studies have not been made after prolonged glucose injections but Table 5 shows the absence of any significant change in adrenal weight in these animals. This is contrasted with the marked increase in adrenal weight in cats treated with crude anterior pituitary extract. The atrophy of the adrenal after hypophysectomy is included in the table for completeness. From Table 4 it is recognized that there is an increased activity of the pituitary adrenal cortical system but as Table 5 shows, this appears to be much less in degree after glucose than after pituitary extract. In this connection it may be noted that exposure to cold, noxious chemicals, etc., which ought to cause the characteristic lowering of adrenal ascorbic acid, failed to increase the glycosuria of diabetic rats in the experiments by Ingle and Nezamis (1943).

The possible local action of intraperitoneal glucose on the pancreas

It is possible that a local action of 20% glucose solution in the peritoneal cavity might have been a factor in causing changes in the islands of Langerhans but the following facts suggest that this is

not the case. (a) The prolonged injection of glucose without 9 or more days of hyperglycemia did not result in island lesions. (b) Hydropic degeneration was present throughout the sections examined and was not confined to the surface of the pancreas as might conceivably occur from local action of the glucose. (c) There were no degenerative changes in the acinar tissue of the pancreas or in other retroperitoneal organs. (d) Only one of the 10 positive cats had evidence of peritonitis whereas 4 of the 25 negative animals had evidence of peritoneal irritation (hyperemia, cloudy fluid) at autopsy. (e) Finally, as Table 6 shows, pancreatic tissue glucose was of similar magnitude after intraperitoneal and intravenous administration of glucose. In these experiments, duplicate samples were taken at 90 to 150 minutes after intra-

TABLE 6. EFFECT OF GLUCOSE ADMINISTRATION ON BLOOD AND PANCREATIC TISSUE GLUCOSE

		Normal cats	After intraperitoneal glucose[a]	After intravenous glucose[b]
		mg. per 100 ml.	mg. per 100 ml.	mg. per 100 ml.
Blood Sugar	Avg.[c]	104 (3)	622 (4)	849 (4)
	Range	93–107	394–937	716–1017
Pancreatic Tissue Glucose	Avg.[c]	35 (3)	178 (4)	145 (4)
	Range	33–39	66–240	107–220

[a] 50 to 100 ml. of 20 per cent glucose in saline. Glucose concentration in peritoneal fluid prior to washing abdominal cavity with saline ranged from 2.8 to 4.0 per cent.
[b] 10 to 20 ml. of 50 per cent glucose in saline.
[c] Figures in parentheses indicate number of animals.

peritoneal administration and at 20 to 90 minutes after intravenous injection of glucose. The abdominal cavity was thoroughly rinsed with warm physiologic saline before obtaining the pancreatic tissue after intraperitoneal glucose. A piece of pancreas (about 0.5 gm.) was then dried with gauze, minced with scissors, placed in 10 ml. of 0.4 per cent tungstic acid. After weighing and filtering, glucose was determined. The increase in tissue glucose in both groups of animals resembles the results of Palmer (1917) in dogs. All the points discussed above lead to the conclusion that the transperitoneal action of glucose on the pancreas is not an important factor in the production of hydropic degeneration of the islets.

Autopsy findings. In the pancreas of animals with hydropic degeneration of the islets, the lesions resembled those described after partial pancreatectomy, pituitary-diabetes or early spontaneous diabetes in the cat (Lande, 1944). Degranulation of the beta cells was the earliest abnormality. As this is sometimes difficult to distinguish, only the unequivocal hydropic degeneration has been reported (Table 1). Only one animal in this series was followed long enough to permit the development of atrophy. There was no inflammatory reaction in the

islands, no changes occurred in the duct cells and hyaline degeneration was not encountered. In the partially depancreatized animals a variable amount of scarring was present as would be expected but there was no evidence that this affected the remaining islands. Three animals had adenomas of islet or duct tissue. the exact nature of the cells being uncertain. One of these was the animal (G-20) which did not become permanently diabetic after 105 days of injection in spite of extreme hydropic change in the islands. Four animals had purulent peritonitis and showed no island lesions. In the others there was no evidence of inflammation of the pancreas from intraperitoneal administration of glucose. All livers sectioned were normal except that of G-16 which was fatty. Kidneys, heart, ovaries, pituitary, thyroid and adrenals were normal by routine stains.

In 5 cats autopsied 1 to 4 days after the beginning of glucose injections and within 4 hours after the last injection, the liver glycogen ranged from 8 to 12%, values which are distinctly above normal. Lower values were found under other conditions. These levels of 8 to 12% glycogen in cats may be compared with 20 to 25% in dogs immediately after intravenous glucose (Butsch, 1934). Incidentally, Pavy (1894) noted that cats had only half as much liver glycogen as dogs after high carbohydrate feeding. Lundsgaard, Nielson and Orskov (1936) reported that perfused dog livers utilized some glucose but that no utilization was observed in cat livers. There is thus some evidence that glucose utilization by the liver may differ in the cat and dog. This is noted in connection with the failure of all workers to maintain prolonged hyperglycemia in the dog and the relative ability of cats to tolerate massive doses of glucose.

DISCUSSION

The role of species, level of blood glucose and duration of hyperglycemia in the production of damage to the islands of Langerhans will be better appreciated if certain previous studies are briefly reviewed. Although there is an extensive literature on the immediate effects of the administration of glucose by various routes, there are relatively few reports of attempts to give large quantities of glucose for long periods of time. In the early experiments, the absence of frequent blood sugar determinations leaves some uncertainty as to the physiological effect produced by the glucose administered. Later studies in which the blood sugar and the condition of the islets have been observed may be reviewed under two general headings.

A. The prolonged administration of glucose with slight hyperglycemia

Verzar and von Kuthy (1930) gave 50 to 150 grams of sugar daily to dogs by stomach tube for as long as 5 months. The fasting blood sugar was only slightly elevated (maximum 128 mgm. per 100 ml.) and glycosuria amounted from a trace to 14 grams per day. However,

glycosuria persisted for as long as a week after stopping glucose, and at the end of the experiments some of the glucose tolerance curves were mildly and temporarily diabetic. Except for the hyperglycemia (galactosemia?) in the course of the production of galactose cataract in rats (Mitchell, 1935) we are unaware that a sustained elevation of the blood sugar level can be produced by orally administered carbohydrate although glycosuria is readily produced. This was recently illustrated by Ingle (1946) who produced alimentary glycosuria in rats. The blood sugar was elevated during the day, hence the glycosuria but it was always normal before the morning feedings. The glycosuria disappeared after a few days in spite of continued increments in diet. The pancreas was not described. Woerner (1938) gave continuous intravenous glucose to guinea pigs for periods up to 28 days. With the exception of a single blood glucose of 270, the hyperglycemia did not exceed 147 mg. per 100 ml. In his later report (Woerner, 1939) he gave larger quantities of glucose. The longest survival was 8 days during which hyperglycemia was 200 mg. per 100 ml. or higher. He notes that "animals receiving 3 plus grams per kilo per hour continuously did not often survive much longer than 2 days." He recognized that "the functional state of the island cells may not always be accurately determined by the granule content of the cells alone," but he nevertheless concluded that when the blood sugar was high the majority of the beta cells showed beginning degenerative changes. The diabetic rat and rabbit have little or no hydropic change in the islands and diabetes in the guinea pig has apparently not been studied.

B. Marked hyperglycemia of short duration

The immediate effect of large amounts of sugar in the blood was reviewed and studied by Evans (1933) who found that single intravenous injections of glucose with marked hyperglycemia were harmless. Butsch (1934) maintained the blood sugar of dogs at very high levels for as long as 51 hours to measure the maximal capacity for glycogen storage. On histological examination the tissues were normal except for the large glycogen deposits in liver and muscle. Rabbits given 300 ml. of 10 per cent glucose per day subcutaneously died in 5 days (Bouckaert, 1934). Paralysis of the hind legs and its improvement when salt was given with glucose were observed. Jacobs and Colwell (1936) gave constant intravenous glucose for periods up to 6 days. However when hyperglycemia was maintained by large doses, the animals usually died within 48 hours. Death was attributed to acidosis, indicated by a marked fall in the CO_2 capacity before death. This was associated with gross hemorrhage in the pancreas and anterior pitutary but no specific lesions of the islands of Langerhans were seen. They described the symptoms preceding death under glucose administration as weakness and ocasional convulsions. Changes in granulation of the beta cells of guinea pigs (Gomori, 1939)

and mice (O'Leary, 1930) have also been seen after single doses of glucose. In dogs, Astwood, Flynn and Krayer (1942) also encountered early death with evidence of hepatic dysfunction revealed by bilirubinemia, impaired bromsulfalein excretion and hemorrhage. In a recent review, Houssay (1942) presents data on dogs given intravenous glucose for 4 days with definite elevation of the blood sugar. At autopsy on the 5th day there were no pancreatic lesions, a result which we would expect at this time interval. In summary, the use of species in which the response of the islands to glucose is uncertain, an inadequate level, or too brief a duration of hyperglycemia have been the chief obstacles in previous studies. In spite of this the careful observation of minor changes has provided a foundation for the experiments described herein.

The mechanism of production of hyperglycemia: comparison with anterior pituitary extract. In the initial development of hyperglycemia the use of glucose differs from that of pituitary extract in many respects. In the first 3 to 4 days, before lesions of the islands appear, crude anterior pituitary extract lowers the R.Q., inhibits the action of insulin on the blood sugar, promotes ketogenesis and thyroid and adrenal hyperplasia occur. There is probably a direct inhibition of the secretion of insulin (Anderson and Long, 1947; Houssay, 1942) and the insulin content of the pancreas is reduced (Best, Campbell and Haist, 1939). In contrast, the administration of glucose initially raises the R.Q., increases the efficiency of injected insulin, is antiketogenic and is not accompanied by thyroid or adrenal hyperplasia. Furthermore, glucose stimulates insulin secretion (Anderson and Long 1947) and carbohydrate diet maintains a high insulin content of the pancreas (Best, Haist and Ridout, 1939). Space does not permit the enumeration of other details. However, these facts suggest that in these two methods of producing hyperglycemia the physiological mechanisms preceding the hyperglycemia are quite different although the later development of hydropic degeneration and diabetes is essentially the same after both glucose and pituitary extract.

The mechanism of island injury. Allen (1913) concluded that the total caloric intake and body mass were important factors in the production of damage to the islets. In our animals with hydropic degeneration of the islands the caloric intake was equal to or less than that of normally fed cats and there was loss of body weight in all but one instance (Table 2). It thus seems that neither excess of calories nor increase in body mass were factors in the production of the lesions of the islands in the present experiments. This does not deny their importance under other conditions. The direct action of intraperitoneal glucose does not appear to be responsible. The diabetogenic property of certain pituitary and adrenal cortical preparations has been demonstrated in recent years and the pituitary-adrenotrophic system has been found to respond to glucose in these experiments. However

the lack of adrenal hypertrophy after glucose and the failure of other stimuli of adrenotrophic activity to aggravate diabetes (Ingle and Nezamis, 1943) lead to the tentative conclusion that this hormonal response is of little importance in the production of lesions. The degree of hyperglycemia and especially its duration have already been emphasized in relation to the development of island lesions. Whether or not there is some agent in addition to hyperglycemia, the events suggest that an excessive functional demand leads to the breakdown of function and to the anatomical changes of the islands. This cycle of functional stress followed by anatomical lesions has been repeatedly demonstrated in at least one other organ, viz. the kidney. The report of Medlar and Blatherwick (1937) is cited as an example of the production of renal insufficiency and renal lesions after unilateral nephrectomy. In the case of the islands, it is clear that hyperglycemia in the living animal is so complex a situation that other mechanisms must still be considered.

SUMMARY

Hydropic degeneration of the islands of Langerhans and persistent diabetes have been produced in cats by the administration of large amounts of glucose. The development of islet lesions was related to the degree and particularly to the duration of hyperglycemia. Neither excessive caloric intake, nor gain in weight, nor the local action of intraperitoneal glucose appear to be responsible. Although the adrenotrophic-adrenal cortical system reacts to intraperitoneal glucose, provisional appraisal of the problem suggests that this is less important than hyperglycemia in the production of islet injury. After hyperglycemia was established the course of the lesions and the development of diabetes closely resembled the sequence of events observed in experimental pituitary-diabetes. The contrast between the effects of glucose and of anterior pituitary extract in the first few days of their administration and the similarity of the events after hyperglycemia develops has been discussed.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Miss Carolyn Doernbach, Miss Marilyn Hess, and of the Messrs. Howard Reese, William Stevenson, Bert Rudin and Herbert Kamins.

REFERENCES

ALLEN, F. M.: Glycosuria and Diabetes. W. M. Leonard, Boston. 1913.
ALLEN, F. M.: *J. Metab. Res.* 1: 5. 1922.
ANDERSON, E., AND J. A. LONG: *Endocrinology* 40: 92. 1947.
ASTWOOD, E. B., J. M. FLYNN AND O. KRAYER: *J. Clin. Investigation* 21: 621. 1942.
BENEDICT, S. R.: *J.A.M.A.* 57: 1193. 1911.
BEST, C. H., J. CAMPBELL AND R. E. HAIST: *J. Physiol.* 97: 200. 1939.
BEST, C. H., R. E. HAIST AND J. H. RIDOUT: *J. Physiol.* 97: 107. 1939.
BLOOR, W. R.: *J. Biol. Chem.* 24: 227. 1916.
BOUCKAERT, J. P.: *Compt. rend Soc. de biol.* 117: 91. 1934.

BUTSCH, W. L.: *Am. J. Physiol.* 108: 639. 1934.
CORI, G. T.: *J. Biol. Chem.* 96: 259. 1932.
DOHAN, F. C., C. A. FISH AND F. D. W. LUKENS: *Endocrinology* 28: 341. 1941.
DOHAN, F. C., AND F. D. W. LUKENS: *Sicence* 105: 183. 1947.
DOUGHERTY, T. F., AND A. WHITE: *Endocrinology* 35: 1. 1944.
EVANS, C.: *J. Physiol.* 77: 189. 1933.
FREEMAN, H., AND F. ELMADJIAN: *J. Clin. Endocrinol.* 6: 668. 1946.
GOMORI, G., N. B. FRIEDMAN AND D. W. CALDWELL: *Proc. Soc. Exper. Biol. & Med.* 41: 567. 1939.
HAIST, R. E., J. CAMPBELL AND C. H. BEST: *New England J. Med.* 223: 607. 1940.
HOUSSAY, B. A.: *Medicina* 2: 3. 1942.
HOMANS, J.: *J. Med. Res.* 30: 49. 1914.
INGLE, D. J.: *Endocrinology* 39: 43. 1946.
INGLE, D. J., AND J. NEZAMIS: *Endocrinology* 33: 181. 1943.
JACOBS, H. R., AND A. R. COLWELL: *Am. J. Physiol.* 116: 194. 1936.
LANDÉ, K. E.: *Am. J. Clin. Path.* 14: 590. 1944.
LONG, C. N. H.: *Fed. Proc.* 6: 461. 1947.
LUKENS, F. D. W., AND F. C. DOHAN: *Endocrinology* 30: 175. 1942.
LUKENS, F. D. W., F. C. DOHAN AND M. W. WOLCOTT: *Endocrinology* 32: 475. 1943.
LUNDSGAARD, E., N. A. NIELSEN AND S. L. ORSKOV: *Skand. Arch. Physiol.* 73: 296. 1936.
MEDLAR, E. M., AND N. R. BLATHERWICK: *Am. J. Path.* 13: 881. 1937.
MITCHELL, H. S.: *Proc. Soc. Exper. Biol. & Med.* 32: 971. 1935.
O'LEARY, J. L.: *Anat. Rec.* 45: 27. 1930.
PALMER, W. W.: *J. Biol. Chem.* 30: 79. 1917.
PAVY, F. W.: The Physiology of the Carbohydrates. J. and A. Churchill, London. 1894.
RAYMOND, A. L., AND J. G. BLANCO: *J. Biol. Chem.* 79: 649. 1928.
ROE, J. H., AND C. A. KUETHER: *J. Biol. Chem.* 147: 399. 1943.
SAYERS, G., AND M. A. SAYERS: *Endocrinology* 40: 265. 1947.
SAYERS, G., M. A. SAYERS, T. Y. LIANG AND C. N. H. LONG: *Endocrinology* 38: 1. 1946.
SELYE, H.: *J. Clin. Endocrinol.* 6: 117. 1946.
SOMOGYI, M.: *J. Biol. Chem.* 160: 61. 1945.
STODDARD, J. L., AND P. E. DRURY: *J. Biol. Chem.* 84: 741. 1929.
VERZAR, F., AND A. VON KUTHY: *Arch. f. d. ges. Physiol.* 225: 606. 1930.
WOERNER, C. A.: *Anat. Rec.* 71: 33. 1938.
WOERNER, C. A.: *Anat. Rec.* 75: 91. 1939.

ORGAN WEIGHTS OF RATS RECEIVING HORMONE SUPPLEMENTS DURING RECOVERY FROM CHRONIC STARVATION

FREEMAN H. QUIMBY

From the Department of Zoology, University of Maryland

COLLEGE PARK, MARYLAND

REDUCED caloric intake has been shown to result in atrophic and functional disturbances of the hypophysis, the resulting reduction in pituitary activity being followed by secondary effects in other endocrine glands and organs. In a review of the literature, Stephens (1941) points out the histological and physiological evidence showing that chronic inanition is accompanied by structural and functional alterations of the pituitary. Both clinical and experimental data indicate that atrophic and degenerative changes in the thyroid, adrenal cortex, gonads, and sex accessories are associated with suppression of the anterior pituitary. Vollmer (1943) emphasizes that the atrophy of some of the endocrine organs in undernutrition is due less to the direct starvation of their tissues than to the failure of hypophyseal support. Trentin and Turner (1941) found that the mammary gland responded to estrogen at low feed levels, but that increased amounts of estrogen were required at such levels because of the physiological suppression of the pituitary during underfeeding. Mulinos and Pomerantz (1940a) summarize the picture by regarding the state of chronic inanition as one of "pseudohypophysectomy."

Although histological changes in the endocrine glands occurring in experimental undernutrition appear to be corrected by refeeding, as shown by Stephens (1940) and Jackson (1917), it does not necessarily follow that normal secretion and function are attained. Normal weight of some of the endocrine glands and other organs are recovered slowly or incompletely. In a study on prolonged refeeding of underfed young male rats, Jackson (1937) found that absolute weights were restored in only the brain, pituitary, kidneys, testes, and epididymides, while weight recovery in the skeleton, thymus, adrenals, heart, liver, and spleen were only relative.

Gonadotrophic effects of pituitary extracts and growth preparations on some of these organs in normal animals have been obtained by Riddle and Polhemus (1931), Friedgood (1936), Lee and Freeman (1940), LeRoy *et al.* (1942), and Hall and Selye (1945). Mulinos and Pomerantz (1941 a and b) employed pituitary implants in adult rats

Received for publication February 12, 1948.

in a state of chronic inanition during continued underfeeding with the result that the thymus was decreased, the testes were increased, and the adrenal glands returned to normal weight. Smith (1930) made daily homeotransplants into hypophysectomized rats with the result that the decreased kidneys, adrenal cortex, thyroid, liver, spleen, and testes were restored or enlarged.

The foregoing constitutes the theoretical basis for the therapeutic use of pituitary extracts as an aid to recovery from inanition. Other endocrine related effects of inanition for which other hormone supplements might prove beneficial is brought out in several investigations. That there is a reduction in gonadotropin in chronic inanition and a consequent decrease in sex hormone secretion has already been shown. The atrophic histological changes in the testes and the atrophy of the accessory sex organs of undernourished rats as described by Mulinos and Pomerantz (1940b–1941c) suggested that injections of testosterone might make a substantial contribution to the recovery of these organs. Vollmer (1943) emphasizes that even though the rat continued to be underfed its genitalia are repaired by gonadotropin or testosterone. Moore and Samuels (1931) corrected the atrophic changes in the prostate and seminal vesicles of undernourished rats by the administration of testosterone. An increase in kidney weight in normal animals following injections of testosterone has been reported by Korenchevsky *et al.* (1933), MacKay (1940), and Luden *et al.* (1941).

Chronic starvation has been found by Jackson (1919) and Mulinos and Pomerantz (1941b) to result in a diminution in the weight of the adrenal glands. Although injections of adrenal cortex extract was not expected to influence the weight of the adrenal glands themselves, the pituitary-cortex and cortex-gonad relationship suggested that cortical supplements might be reflected in the weights of other glands.

The purpose of this investigation was (1) to check the work of Jackson (1915, 1925, 1937), Stewart (1916, 1918), and Stefko (1927) on the alterations in organ weights during chronic starvation and recovery, and to give analysis and interpretation to these changes, and (2) to determine the effects of supplements of adrenal cortex extract, testosterone, and growth hormone on organ weights, administered during realimentation and recovery.

METHODS

The studies made in this work were arranged in two separate experiments. In both experiments young male albino rats 30 days of age were employed in groups of ten, each group constituting an experimental unit. The rats were placed in individual cages and were underfed by restriction to a constant daily body weight ration of a qualitatively balanced diet. At the end of the selected periods of underfeeding the animals were put upon full and adequate rations for various periods of time. During recovery the supplements were adminsitered daily by intraperitoneal injection. At the close

of the selected refeeding periods the animals were anesthetized by nembutal, exsanguinated, and autopsied. In Experiment 1 each rat was underfed for 30 days and then refed for 35 days, receiving during the recovery a schedule of therapeutic injections as follows: Group I, 20 rat units—Growth Hormone ("Phykentrone," Squibb), Group II, 20 dog units—"Adrenal Cortex Extract" (Upjohn), Group III, ½ ml.—"Physiological Salt Solution" (Lilly). In Experiment II each rat was underfed for 90 days and then refed 60 days, receiving during the recovery a schedule of therapeutic injections as follows: Group IV, 5 rat units—"Growth Hormone." Group V, ½ mg.—testosterone "Oreton" (Schering). Group VI, ½ ml.—"Physiological Salt Solution" (Lilly).

In addition to these groups of refed rats, two groups of fully fed controls were observed during recovery; one was the same weight as the starved rats, and the other was the same age as the starved rats.

TABLE 1. AVERAGE ABSOLUTE WEIGHTS OF THE ORGANS OF RATS WHICH WERE CHRONICALLY STARVED AND THEN REFED AND INJECTED WITH HORMONE SUPPLEMENTS
(Grams)

Experimental group	Pituitary	Adrenal	Brain	Spleen	Kidney	Testis	Epididymides	Seminal vesicles	Thymus	Heart	Liver
Underfed 30 days	.0028	.0100	1.351	.212	.330	.390	.164	.160	.041	.217	2.40
Refed 35 days											
Saline	.0116	.0163	2.295	.705	1.032	1.237	1.010	.473	.417	.777	8.70
Cortex	.0116	.0156	2.260	.734	1.148	1.256	.994	.405	.419	.908	10.22
Growth	.0116	*.0246	2.219	*.844	*.966	1.103	*.726	* .334	*.627	.782	9.87
Normal control	.0139	.0205	2.403	1.355	1.164	1.354	1.200	.481	.370	1.025	11.65
(Percentage weight on body weight)											
Underfed 30 days	.0048	.0172	2.620	.362	.564	.669	.273	.266	.070	.370	4.10
Refed 35 days											
Saline	.0049	.0067	.960	.296	.434	.520	.425	.199	.175	.327	3.66
Cortex	.0047	.0063	.940	.297	.465	.510	.405	.164	.169	.368	4.14
Growth	.0049	*.0103	.933	*.352	*.405	.460	*.303	*.140	*.262	.329	4.15
Normal control	.0047	.0070	.815	.460	.396	.460	.410	.164	.147	.350	3.96

* Indicates statistical significance.

In order to obtain data relevant to chronic starvation itself measurements covering all phases of this investigation were made on a group of rats sacrificed at the end of the starvation period.

After removing the blood as described above, the organs and glands were dissected out and their fresh weights determined on an analytical balance. The larger organs were weighed in regular weighing vials, but the adrenal glands, pituitary, and the thymus and seminal vesicles of the small normal rats and the underfed rats were weighed in vials of fixative the tare weight of which had been predetermined.

RESULTS

The numbers in the tables represent averages of a group of ten determinations on ten rats. The reliability of the averages was determined by plotting the range and mean deviation. Only differences that were found significant by this method are considered in the discussion.

The absolute and relative organ weights in chronic starvation, recovery, and recovery therapy are summarized in Tables 1 and 2.

DISCUSSION

The Effect of Chronic Starvation on Organ Weights. The effects of chronic inanition on the relative organ weights as determined by this experiment were for the most part the same as those found by Jackson (1915). There was no change in the average weight of the brain, an increase in the weight of the testes and pituitary, and a decrease in the weight of the thymus and spleen. The effects on the spleen and thymus as shown by these studies on rats are in accord with those observed on children by Stefko (1927). Disagreement appears regarding only the kidneys and liver, Jackson (1915) recording a slight increase in the weights of these organs, and this experiment showing no significant change in the kidneys and a decrease in the liver. While the

TABLE 2. AVERAGE ABSOLUTE WEIGHTS OF THE ORGANS OF RATS WHICH WERE CHRONICALLY STARVED AND THEN REFED AND INJECTED WITH HORMONE SUPPLEMENTS

(Grams)

Experimental group	Kidney	Adrenal	Thymus	Brain	Testis	Seminal vesicle	Spleen	Liver	Pituitary	Heart
Underfed 90 days	.334	.0094	.026	1.41	.325	.015	.203	2.07	.0030	.20
Refed 60 days										
Growth	1.026	*.0225	.361	1.72	1.054	.472	.679	10.00	.0101	.83
Testosterone	*1.262	.0139	*.172	1.69	*.821	*1.122	.609	11.10	.0085	.95
Saline	1.308	.0165	.411	1.80	1.287	.548	.726	12.30	.0100	.93
Normal control (same weight)	.347	.0080	.179	1.34	.156	.032	.299	2.65	.0021	.28
Normal control (same age)	1.075	.0201	.309	1.80	1.304	.529	.962	12.60	.0119	1.06
				(Percentage weight on body weight)						
Underfed 90 days	.605	.0170	.472	2.560	.590	.027	.363	3.76	.0054	.362
Growth	.431	*.0096	.1506	.724	.428	.192	.284	4.18	.0043	.349
Testosterone	*.532	.0059	*.0723	.713	*.350	*.581	.256	4.56	.0032*	.381
Saline	.482	.0062	.1519	.673	.480	.201	.273	4.62	.0037	.343
Normal control (same weight)	.650	.0150	.3360	2.522	.291	.059	.562	4.95	.0039	.523
Normal control (same age)	.454	.0067	.1137	.605	.439	.177	.328	.422	.0041	.355

* Indicates statistical significance.

difference in results on kidney weights is likely due to the age of compared controls, that on the liver is more difficult to explain. The reduced liver weights of this experiment, however, agree with those found by Stefko (1927) in underfed children. The weight of the adrenal glands in the underfed rats was in nearly the same ratio to the body weight as that of normal rats of the same size, but is increased and consequently in accord with Stewart (1918) when compared with controls of the same age. Since the relative weight of the adrenals decreases with age in normal animals, this latter comparison is logical. The relative weight of the heart is also reduced with body growth so that when compared with controls of the same age there was no significant change as a result of starvation. On the other hand, the weight of the heart was relatively less as a result of underfeeding when compared with normal controls of the same size. Stefko (1927) observed a similar reduction in heart size in children suffering from chronic malnutrition. Consideration given to the age and size of the control rats does not otherwise alter the general effects of underfeeding as already

stated, except in the case of the brain. The brain of an animal starved in any way at any age always constitutes a greater per cent of the body weight than it does in a normal animal of the same age.

Since the weights of the kidney, brain, pituitary, adrenal glands, and testes in chronic starvation were in normal or greater than normal proportion to the body weight, it is concluded that these organs were "preserved" or "protected" during underfeeding in young rats. Probably the growth impulse of these organs overbalances the effects of caloric restriction.

The thymus, seminal vesicles, spleen, and heart, on the other hand, sacrifice their growth and contribute their substance in a proportion greater than other organs and body tissues. The reduction of the thymus is probably a case of vitamin involution, since Stoerk and Zucker (1944) found that in otherwise adequate diets, partial deficiency of vitamins of the B complex depressed the thymus weight. Sure (1938) also concludes that in B_1 deficiency there is atrophy of the thymus. Reduction of the seminal vesicles in the chronic starvation of these rats is due to the diminished male sex hormone in connection with the inhibited testes. Mulinos and Pomerantz (1941c) have employed this interpretation to similar results in adult rats.

The relatively greater weight of the testes and pituitary at the end of the underfeeding period shows that these organs continued to grow slowly during starvation in spite of the suspended growth of the body and other organs. In view of the depressing effects of chronic inanition on the pituitary gland of adult rats, as shown by Mulinos and Pomerantz (1940a), and more specifically in view of the disturbed protein metabolism and reduced metabolic rates which occurred in the underfed rats of this investigation, one might have expected the pituitary to have had relatively less weight than the body. This is particularly true if consideration is given to the atrophic histological picture of the pituitary in chronic inanition in young rats described by Jackson (1917). However, an enlarged gland is not necessarily an active or over-active gland, as seen in the familiar response of the thyroid to endemic iodine deficiency; and the relative hypertrophy of the pituitary in the starved rats of this study may be just as indicative of a disturbed and inhibited function as if the gland had been reduced in size and weight.

The fact that the testes exhibited a marked weight gain in the presence of caloric restriction strongly suggests that the growth impulse in these organs was not independent of pituitary support. It may be, therefore, that the pituitary of young rats in chronic inanition continues to secrete a physiologically effective though reduced amount of gonadotropic hormone. A further evidence of some gonadotropic secretion may be found in the fact that in over 200 young male rats, chronically starved from 30 days' age, there were only two instances of an undescended testis.

Another possible instance of continued pituitary support is in the case of the adrenotropic hormone. While chronic inanition in adult rats is accompanied by an adrenal atrophy, as shown by Mulinos and Pomerantz (1941b) a marked adrenal hypertrophy occurred in the young rats of this investigation when compared with normal animals of the same age. It cannot be argued that the growth impulse prevents the adrenal atrophy in young rats, because the relative weight of the adrenal glands decreases with normal body growth.

The increases in the relative weights of the pituitary and adrenal glands during chronic underfeeding may be attributed to the lack of vitamin B_1 in the reduced daily ration. This explanation is proposed in view of the work of Sure (1938) who found that B_1 deficiency caused a marked hypertrophy of both of these organs.

The Effects of Refeeding on Organ Weights. The results of refeeding on the weights of organs corroborate those of Stewart (1916), with normal recovery of the brain, kidneys, and testes. There is also agreement on the relative recovery hypertrophy of the thymus and liver. The relative hypertrophy of the spleen in recovery as observed by Jackson (1925), however, is not confirmed by this experiment. Disagreement on this point is probably due to the tremendous individual variation in the weight of the spleen.

The recovery of the relative weights of the pituitary and adrenal glands as reported by Jackson (1937) in prolonged refeeding was observed in this experiment in rats underfed for 30 days, but was not observed in those underfed for 90 days. It appears therefore that the extent to which these two organs are restored depends upon the length of the undernutrition period.

While most of the effects of chronic inanition on young male rats are reversible, it is clear that these animals are not free from the stigma of the underfed condition. The effect on the weights of the kidneys, brain, testes, seminal vesicles, liver, and heart appear to be completely reversible, since these organs attain a normal absolute weight. The effects on the body weight, body growth, the weight of the adrenal glands, thymus, spleen, and pituitary are, on the other hand, only partially reversible since the recovery of these values was either lacking entirely or only relative to the partially recovered body weight.

The Effect of Growth Hormone on Organ Weights. An increase in the weight of the adrenal glands was affected by both small and large injections of growth hormone during recovery. This may have been due to traces of adrenotropic hormone which were probably present in the preparation employed. Friedgood (1936) and Hall and Selye (1945) obtained similar results with growth extracts on normal animals. The hypogenic effect on the adrenals obtained by Klieber and Cole (1939) with Evans' growth preparation may have been due to the very long period over which injections were made.

Large injections of growth hormone during recovery from the shorter starvation period produced enlargement of the spleen. This result is interpreted as indicating a "splenotropic" action on the part of the growth preparation used. Friedgood (1936) also noted an increase in the weight of the spleen in normal guinea pigs treated with an alkaline pituitary extract. However, using a similar extract, Riddle and Polhemus (1931) found no significant change in the spleen of young pigeons.

The growth hormone in large doses decreased the weight of the testes and sex accessories. This effect is interpreted as due to the inhibiting action on the pituitary gland of the growth hormone in general, and of the adrenocorticotropic hormone in particular. It is axiomatic that hormone supplements inhibit the gland which normally produced the hormone. Koneff (1944) has recently shown that the adrenocorticotropic hormone inhibits the anterior pituitary. Korenchevsky (1930) obtained a similar reduction in testes and sex accessories in normal animals with an alkaline pituitary extract.

A decrease in the weight of the kidneys resulted from the large injections of growth hormone. No measurements taken in this experiment and no published studies of a related nature made it possible to interpret this effect. In view of the results it can only be stated that the growth preparation used has an "anti-renotropic" action. This effect of the growth hormone on young rats recovering from inanition is in contrast with the kidney enlargement obtained with a crude pituitary extract on normal animals by Hall and Selye (1945).

No significant alteration in either the heart or liver appeared as a result of growth hormone administration. Other pituitary preparations have produced effects on normal animals which vary on either side of those obtained in this experiment. For example, extracts used by Lee and Freeman (1940) and by Riddle and Polhemus (1931) produced a liver hypertrophy, while those employed by Klieber and Cole (1939) and by LeRoy *et al.* (1942) resulted in liver reduction. Similarly, Hall and Selye (1945—report an enlargement of the heart with their extract, while Kliever and Cole (1939) found a reduction in heart size using Evans' extract. These disagreements undoubtedly arise from differences in the extracts, methods, and conditions employed.

Thus, while the growth hormone in heavy doses tended to increase and restore the normal weight of the spleen, it at the same time decreased the weights of the kidneys, testes, and sex accessories, and produced an abnormal hypertrophy of the adrenal glands. These results make it clear that large amounts of growth preparations are not indicated in recovery from chronic inanition. Since light injections of growth hormone, even after prolonged starvation, restored the normal weight of the adrenal glands without producing undesirable alterations in other organs, it may be that small amounts of prepara-

tions containing the adrenotropic hormone would be useful in effecting an earlier cortical recovery.

The Effect of Testosterone on Organ Weights. The testes were decreased in weight as a result of injections of testosterone during recovery. This effect was probably mediated through the inhibiting action of testosterone on the gonadotropic function of the pituitary gland. The interpretation has been proposed by Selye and Friedman (1941) and Shay *et al.* (1941) for a similar effect of testosterone on normal animals. There is also the possibility that the depressing action of testosterone injections on the testes may have been due to the oil vehicle rather than to the hormone itself. Cameron, Guthrie, and Carmichael (1946) found that daily injections of peanut oil for 17 or 18 days caused testicular atrophy and a decrease in rate of growth in the rat.

Testosterone administration produced a marked enlargement of the seminal vesicles; and since as stated above the testes themselves were inhibited by testosterone, it must be that the effect was due to the stimulating action of the hormone directly on the seminal vesicles.

A marked atrophy of the thymus occurred in rats treated with testosterone during refeeding. This effect was to be expected on the basis of the thymic involution which occurs normally in animals after puberty. Korenchevsky *et al.* (1933) obtained similar reduction of the thymus in normal animals injected with testosterone.

Rats receiving supplements of testosterone during recovery exhibited a significant increase in the weight of the kidneys. This action has also been observed on normal animals by Korenchevsky *et al.* (1933), MacKay (1940), and Luden *et al.* (1941). The mechanism and significance of this effect remains obscure.

The use of testosterone during recovery was attended with no measurable advantages, while presumably there were the undesirable effects of abnormal hypertrophy of the sex accessories, excessive thymic involution, and depression of the testes. It may be concluded therefore, that the administration of the male sex hormone in the amounts employed in this experiment is without benefit, if not actually contraindicated.

The Effects of Adrenal Cortex Extract on Organ Weights. Since the effects of the adrenal cortex extract on the thymus, seminal vesicles, and adrenal glands were very slight, the results are interpreted to be without significance. There may be in these small changes, however, a further evidence of the adrenal-gonad relationship.

The cortex extract produced no alterations in the weights of the organs which would justify either its use or disuse in recovery from inanition.

SUMMARY

Chronic starvation of both 30 and 90 days' duration decreased in young male rats the relative weights of the liver, thymus, heart,

seminal vesicles, and spleen; increased the relative weights of the adrenal glands, testes, and pituitary; and produced no change in the absolute weights of the brain and kidneys. The alterations produced were such that although refeeding resulted in the restoration of normal weights for the seminal vesicles and testes; the spleen, pituitary and adrenals failed to recover a weight in normal proportion to the body and the liver and thymus showed a relative hypertrophy.

The purpose of this investigation was to determine the effects of therapeutic supplements of adrenal cortex extract, growth hormone, and testosterone administered during recovery from chronic starvation upon the final organ weights of the rat.

One hundred rats, 30 days old and 50 grams weight, were brought to a state of chronic inanition by a qualitatively balanced constant body weight ration. They were then refed and groups of ten were injected with a different hormone. Two different periods of starvation and recovery were studied. Observations were made on body and organ weights on both starved and refed animals, as well as on the fully fed controls. All results were expressed as an average of ten animals.

The growth hormone administered during refeeding effected a normal absolute weight for the adrenals, but dosages adequate to increase also the weight of the spleen had a depressing effect on the testes, seminal vesicles ,and kidneys.

Injections of testosterone, while increasing the weights of the kidneys and seminal vesicles, decreased the weights of the thymus and testes.

The adrenal cortex extract produced no alteration in organ weights which could be regarded as significant.

REFERENCES

CAMERON, A. T., J. S. GUTHRIE AND J. CARMICHAEL: *Canadian J. Research Sect. E. Med. Sci.* 24: 105. 1946.

FRIEDGOOD, H. B.: *Endocrinology* 20: 159. 1936.

HALL, C. R., AND H. SELYE: *Rev. Canadienne Biol.* 4: 197. 1945.

JACKSON, C. M.: *J. Exper. Zool.* 19: 99. 1915.

JACKSON, C. M.: *Am. J. Anat.* 21: 321. 1917.

JACKSON, C. M.: *Am. J. Anat.* 25: 221. 1919.

JACKSON, C. M.: *Inanition and Malnutrition.* Philadelphia: P. Blakiston & Co., 1925.

JACKSON, C. M.: *Anat. Rec.* 68: 371. 1937.

KLEIBER, M., AND H. H. COLE: *Amer. J. Physiol.* 125: 747. 1939.

KONEFF, A. A.: *Endocrinology* 34: 77–82. 1944.

KORENCHEVSKY, V.: *Biochem. J.* 14: 383. 1930.

KORENCHEVSKY, V.: *Biochem. J.* 27: 557. 1933.

LEE, M., AND W. FREEMAN: *Endocrinology* 26: 493. 1940.

LEROY, V., M. KRISS, L. F. MARCY AND R. S. BOWMAN: *J. Nutrition.* 24: 469; 24: 481. 1942.

LUDDEN, J. B., E. KRUEGER AND I. S. WRIGHT: *Endocrinology* 28: 519. 1941.

MACKAY, E. M.: *Proc. Soc. Exper. Biol. & Med.* 45: 216. 1940.

MULINOS, M. G., AND L. POMERANTZ: *J. Nutrition* 19: 493. 1940a.

MULINOS, M. G., AND L. POMERANTZ: *J. Nutrition* 19: 943. 1940b.

MULINOS, M. G., AND L. POMERANTZ: *Endocrinology* 29: 558. 1941a.

MULINOS, M. G., AND L. POMERANTZ: *Amer. J. Physiol.* 132: 368. 1941b.
MULINOS, M. G., AND L. POMERANTZ: *Endocrinology* 29: 267. 1941c.
RIDDLE, O., AND I. POLHEMUS: *Amer. J. Physiol.* 98: 121. 1931.
SELYE, H., AND S. FRIEDMAN: *Endocrinology* 28: 129. 1941.
SHAY, H., J. G. COHEN, K. PASCHKIS AND S. S. FELS: *Endocrinology* 28: 877. 1941.
SMITH, P. E.: *Am. J. Anat.* 45: 205. 1930.
STEFKO, W. H.: *Ergebn. d. allg. Path. u. path. Anat.* 22: 687. 1927.
STEWART, C. A.: *Biol. Bull.* 31: 16. 1916.
STEWART, C. A.: *J. Exper. Zool.* 25: 301. 1918.
STEPHENS, D. J.: *J. Clin. Endocrinol.* 1: 256. 1941.
STOERK, H. C., AND T. F. ZUCKER: *Proc. Exper. Biol. & Med.* 56: 151. 1944.
SURE, B.: *Endcrinology* 23: 575. 1938.
TRENTIN, J. J., AND C. W. TURNER: *Endocrinology* 29: 984. 1941.
VOLLMER, E. P.: *The Scientific Monthly.* 438. 1943.

EFFECT OF VITAMIN DEFICIENCIES IN NEW HAMPSHIRE CHICKS INJECTED WITH HIGH DOSES OF THYROXINE[1,2]

M. E. HAQUE, R. J. LILLIE, C. S. SHAFFNER AND G. M. BRIGGS[3]
From the Poultry Department, University of Maryland
COLLEGE PARK, MARYLAND

HERTZ AND SEBRELL (1944) observed an interesting example of the interdependence of a dietary trace substance and a hormone-like factor; that is, between the metabolism of estrogens and the vitamin B complex, and further emphasized the importance of adequate nutrition for normal endocrine function. Hertz (1945) studied the direct quantitative relationship between the degree of oviduct response to stilbestrol and the dietary level of folic acid.

The vitamin requirements of hyperthyroid animals appear to be increased. In addition to the observations of Abelin *et al.* (1930), Abelin (1933), and von Euler and Klussman (1932) as quoted by Betheil *et al.* (1947), the following workers obtained evidence of increased requirements of vitamin A in the rat during experimental hyperthyroidism: Logaras and Drummond (1938), Sure and Buchanan (1937b). The same was found to be true for ascorbic acid requirements in the guinea pig by Svirbely (1935) and Sure and Theis (1939).

It may be assumed that an increased metabolic rate which results from the administration of a thyroid material may necessitate an increased dietary intake of members of the B complex. The results of many workers confirm such a hypothesis (Abelin, 1930) as quoted by Betheil *et al.* (1947), Cowgill and Palmieiri (1933), Himwitch *et al.* (1931, 1932). The partial protective effect against thyrotoxicity exerted by vitamin B_1 was first shown by Sure and Smith (1934), Sure and Buchanan (1937a) and confirmed by Drill and Sherwood (1938). Drill and Overman (1942) found that pyridoxine and Ca-pantothenate were required in larger quantities during experimentally induced thyrotoxicosis. Abelin (1945, 1946), as quoted by Betheil *et al.* (1947), feeding a crude ration, observed a beneficial effect when massive doses of Ca-pantothenate were administered to hyperthyroid rats. Ershoff and Hershberg (1945) were unable to alleviate the symptoms resulting from thyroid administration by greatly increasing the

Received for publication February 16, 1948.
[1] Scientific Paper No. A194. Contribution No. 2107 of the Maryland Agricultural Experiment Station (Department of Poultry Husbandry).
[2] This study was supported in part by a grant from Ciba Pharmaceutical Products, Inc., Summit, New Jersey.
[3] Present address: Poultry Section, University Farm, St. Paul 1, Minnesota.

Ca-pantothenate level of the ration. Ershoff and Hershberg (1945) found that rats fed a purified ration very high in the members of vitamin B complex known to be required by the rat grew poorly when desiccated thyroid was added. Betheil *et al.* (1947) have shown that very high levels of Ca-pantothenate as well as inositol exerted no beneficial effect on thyrotoxicosis.

Our object was to investigate further the relationships between vitamins and hormones with similar studies. The present experiment was planned to study the effects of various vitamin deficiencies on response to injected thyroxine.

EXPERIMENTAL PROCEDURE

Day-old New Hampshire chicks were reared in electrically heated batteries with raised screen floors. Feed and water were given *ad libitum*. The chicks were fed the respective diets for the full 4 weeks of the trials, body weights being recorded at weekly intervals. Table 1 gives the complete

TABLE 1. COMPOSITION OF BASAL RATION 110

Main ingredients	Per cent	Supplements	Mg./100 gm.
Glucose ("Cerelose")	61.4	Thiamine HCl	0.4
Casein (crude)	18.0	Riboflavin	0.8
Gelatin	10.0	Ca pantothenate	2.0
Soybean oil[1]	4.0	Nicotinic acid	5.0
Salts IM (Briggs, 1946)	6.0	Choline Cl	200.0
L cystine	0.3	Pyrixodine HCl	0.6
		Biotin	0.02
		Folic acid	0.2
		i-Inositol	100.0
		Para-aminobenzoic acid	0.2
		Alpha-tocopherol	0.5
		2-Methyl-1, 4-naphthoquinone	0.1
		Vitamin A in I.U.	1200[2]
		Vitamin D_3 in A.O.A.C. units	170[2]

[1] Substituted with Majola oil in the 3rd trial.
[2] Vitamins A and D_3 are fed by dropper weekly.

ration (110) used. The vitamin deficient rations were made either by completely omitting the vitamin concerned or by adding a low quantity as shown in Table 2. A low quantity of vitamin was added to the ration when necessary to prevent excessive mortality. At the end of 3 weeks, observations were made for the presence of naturally occurring black in the feathers. At that time a single injection of thyroxine was given.

The thyroxine solution was made such that one ml. of solution contained two mg. of crystalline thyroxine. The chicks were given one intramuscular injection of 0.5 ml. of thryoxine solution per 100 gms. of body weight on the 21st day. Thyroxine injections into New Hampshire chicks cause blackening at the base of the wing feathers (Juhn and Bernes, 1931; Juhn, 1933). This fact was the basis of the observations made on the response of thyroxine as affected by vitamin deficiencies. The incidence of mortality of chicks was recorded daily after injection on the 21st day. The records of blackening of the feathers were made on the 28th day. The blackening of the feathers was measured by the depth and width of melanin deposition in the feathers.

RESULTS

The results of all the experiments are summarized in Table 2. The typical deficiency symptoms of every vitamin were present in the respective group. The results obtained after several repetitions of the thyroxine treated and untreated chicks fed a ration deficient in different vitamins can be grouped as follows:

Feather-blackening.—Twenty-three out of 25 chicks of group 2 (complete ration—thyroxine injected) showed blackening at the base of the secondary and in some cases, primary feathers, which is the

TABLE 2. EFFECT OF VITAMIN DEFICIENCIES IN CHICKS INJECTED WITH THYROXINE

Group	Ration	No. chicks at 3 wks.	No. chicks at 4 wks.	Mortality	Average body weight		Gain, 3–4 wks., gm.	No. of black feathers		Increase of black feathers at 4 wks.
					3 wks. gm.	4 wks. gm.		3 wks.	4 wks.	
1	110 (complete diet)	26	26	0	222	291	70	2	2	0
2	110 +thyroxine (injected)	25	25	0	235	313	78	0	23	23
3	110 with low folic acid (15 gamma)*	15	12	3	113	101	−12	1	1	0
4	As group 3 +thyroxine	14	6	8	113	184	71	0	2	2
5	110 with low pantothenic acid (0.15 mg.)*	16	13	3	123	140	17	0	2	2
6	As group 5 +thyroxine	15	7	8	116	161	45	0	6	6
7	110 with low riboflavin (0.1 mg.)*	10	10	0	94	105	11	0	0	0
8	As group 7 +thyroxine	9	9	0	109	123	14	0	8	8
9	110 minus vitamin D	10	10	0	209	246	37	0	10	10
10	As group 9 +thyroxine	10	9	1	178	234	56	0	9	9
11	110 minus biotin	7	4	3	176	239	63	0	0	0
12	As group 11 +thyroxine	8	6	2	177	221	44	0	2	2
13	110 minus vitamin A	4	2	2	174	148	−26	0	0	0
14	As group 13 +thyroxine	4	0	4	170	—	—	0	0	0
15	110 minus vitamin E	8	8	0	210	257	47	2	6	4
16	As group 15 +thyroxine	8	8	0	206	251	45	0	8	8
17	110 with low B_1 (100γ)*	6	5	1	198	197	− 1	2	2	0
18	As group 17 +thyroxine	6	4	2	161	207	46	0	3	3
19	110 with low B_6 (100γ)*	7	7	0	145	188	43	0	0	0
20	As group 19 +thyroxine	8	6	2	133	172	39	0	6	6

* Amount per 100 grams of ration.

normal response of heavy thyroxine injection. Thyroxine injection of birds in lot 6 (low in pantothenic acid), lot 8 (low in riboflavin), lot 12 (deficient in biotin), lot 18 (low in B_1), and lot 20 (low in B_6) produced blackening of the feathers in a majority of birds. In groups 3 and 4 (low in folic acid), only 2 out of 6 birds had black feathers due to thyroxine injections. This low incidence may be partially accounted for by the slow rate of feathering caused by the deficiency of folic acid. Birds of both lots 9 and 10 (deficient in vitamin D) showed blackening of feathers, as has been reported by Glazener *et al.* (1946). However, the pigmentation of the feathers of birds from lot 10 (vitamin D deficient plus thyroxine) was of deeper intensity, distinct and in streak form whereas that of group 9 (vitamin D alone) was lighter and scattered. This experiment further indicates that a deficiency of vitamin E may produce blackening of feathers in itself. The thyroxine-treated group 16 (vitamin E deficient) showed blackening in the feathers similar to group 10 (vitamin D deficient plus thyroxine). In groups 13 and 14 (vitamin A deficient), incidence of feather blackening could not be recorded due to the death of all injected birds.

Mortality.—Birds of groups 3 and 4 were fed a ration low in folic acid. On thyroxine injection 8 out of 14 chicks died after the thyroxine injection. The pantothenic acid deficient chicks acted similarly, since 8 out of 15 thyroxine-treated birds died. Weaker birds died, leaving stronger ones in both the above groups, which probably accounts for the increase over the controls in average 4-week body weight noted below.

Body Weights.—It will be noted in Table 2 that in the first 5 series of comparisons, including the control lot, there is a consistent increase in gain of weight by the birds receiving the thyroxine injections. A total of 77 uninjected and 73 thyroxine-injected birds are involved in these comparisons. Although the differences between treated and untreated chicks by lots are not statistically significant, there is a consistent increase in favor of those lots receiving thyroxine.

In the remaining lots, the number of birds alive at the end of the experiment is too small to give any value to this measure.

Other Observations.—Another interesting effect of thyroxine injection was observed in chicks fed a ration low in pantothenic acid, in which a marked decrease in the deficiency symptoms of this vitamin (lot 6) was seen. Dermatitis observations were made during the second trial. Of the 6 surviving chicks of the uninjected group, all showed severe dermatitis. Of the 5 surviving injected chicks, only 2 showed mild dermatitis. Possibly the small amount of dermatitis which appeared might be eliminated entirely by the use of different levels of thyroxine, although there is no direct evidence for this as yet.

Further evidence for the effectiveness of thyroxine treatment was found in the fact that upon weighing the thyroid glands at autopsy those birds having received thyroxine consistently had smaller thyroid glands.

The average thyroid weight, calculated on the basis of mgm. per 100 gm. of body weight, was 8.38 mgm. for the controls and 4.65 mgm. for the treated group.

DISCUSSION

The data presented here show that thyroxine produces blackening of feathers in New Hampshire chicks even regardless of a deficiency of the following vitamins: pantothenic acid, riboflavin, thiamin, pyridoxine, and biotin.

The blackening of feathers produced in thyroxine-treated chicks fed a ration deficient in vitamins D and E was deeper than was produced in vitamin D- and E-deficient uninjected groups. This indicates that thyroxine metabolism was not affected in the absence of the above-mentioned B complex vitamins. In the absence of vitamins D and E, injected thyroxine activated the melanin cells to an even greater extent to deposit more black pigment in the feathers. In a majority of the thyroxine-treated chicks fed a ration low in folic acid there was no blackening. This may be accounted for in two ways—

either it was due to the slow rate of feathering or to the possibility that thyroxine metabolism was affected either directly or indirectly. As another possibility, perhaps the melanin-producing cells were affected directly by the folic acid deficiency.

In groups 4 and 6 (low in folic acid and pantothenic acid), higher mortality was observed as a result of thyroxine injection. For some reason the vitamin deficient chicks were susceptible to thyroxine toxicity. Therefore, in chicks these two vitamins appear to be needed in order to counteract the toxic effects of thyroxine.

The above results may be explained by the work of Abelin (1946) as quoted by Betheil *et al.* (1947), who showed a definite relationship between pantothenic acid and thyroxine in rats. He stated that thyroxine influences nearly all cells where pantothenic acid is present. This may possibly explain why injected thyroxine appeared to increase the body weights of some of the other groups (which had pantothenic acid in the ration).

The marked decrease in the deficiency symptoms of pantothenic acid in chicks, as a result of thyroxine injection, is another interesting vitamin and hormone relationship.

SUMMARY

In New Hampshire chicks fed a ration low in thiamin, pyridoxine, biotin, pantothenic acid, and riboflavin, injected thyroxine produces blackening at the base of the wing feathers. In vitamins D and E deficiency, thyroxine produced deeper blackening than was observed in D and E deficiency without thyroxine. Thyroxine caused high mortality in chicks fed a ration low in folic acid and pantothenic acid. Pantothenic acid deficiency symptoms in chicks were partially counteracted by thyroxine injection.

ACKNOWLEDGMENTS

The authors are indebted to Dr. D. G. Perkins, Manager, Veterinary Division, E. R. Squibb & Sons, New York, for crystalline thyroxine; E. I. du Pont de Nemours & Co., New Brunswick, New Jersey, for vitamin D_3, and Dr. M. A. Jull for valuable suggestions.

REFERENCES

Betheil, J. J., V. D. Wiebelhaus and H. A. Lardy: *J. Nutrition* 34: 431. 1947.
Briggs, G. M.: *J. Nutrition* 31: 79. 1946.
Cowgill, G. R., and M. L. Palmieri: *Am. J. Physiol.* 105: 146. 1933.
Drill, V. A., and C. R. Sherwood: *Am. J. Physiol.* 124: 683. 1938.
Drill, V. A., and R. Overman: *Am. J. Physiol.* 135: 474. 1942.
Ershoff, B. H., and D. Hershberg: *Am. J. Physiol.* 145: 16. 1945.
Glazener, E. W., J. P. Mattingly and G. M. Briggs: *Poul. Sci.* 25: 1. 1946.
Hertz, R., and W. B. Sebrell: *Science* 100: 293. 1944.
Hertz, R.: *Endocrinology* 37: 1. 1945.
Himwitch, H. E., W. Goldfarb and G. R. Cowgill: *Proc. Soc. Exper. Biol. & Med.* 28: 646. 1931.
Himwitch, H. E., W. Goldfarb and G. R. Cowgill: *Am. J. Physiol.* 99: 689. 1932.

JUHN, M., AND B. O. BERNES: *Am. J. Physiol.* 98: 463. 1931.
JUHN, M.: *Endocrinology* 17: 88. 1933.
LOGARAS, G., AND J. DRUMMOND: *Biochem. J.* 32: 964. 1938.
SURE, B., AND M. E. SMITH: *J. Nutrition* 7: 547. 1934.
SURE, B., AND K. S. BUCHANAN: *J. Nutrition* 13: 513. 1937a.
SURE, B., AND K. S. BUCHANAN: *J. Nutrition* 13: 521. 1937b.
SURE, B., AND R. M. THEIS: *Endocrinology* 24: 672. 1939.
SVIRBELY, J. L.: *J. Biol. Chem.* 111: 147. 1935.

THE ROLE OF BILE IN THE ABSORPTION OF STEROID HORMONES FROM THE GASTRO-INTESTINAL TRACT[1]

M. M. HOFFMAN, G. MASSON[2] AND M. L. DESBARATS

From the McGill University Clinic, Royal Victoria Hospital, and the Department of Anatomy, McGill University

MONTREAL, CANADA

SINCE BILE is required for the optimum absorption of cholesterol from the gastro-intestinal tract (Schonheimer, 1924) it has been assumed that bile is also essential for the enteral absorption of steroid hormones and related substances. On this assumption bile salts have been administered concurrently with steroid hormones to patients and experimental animals in an endeavor to enhance the potency of the latter substances when they are administered orally. Silberman, Radman and Abarbanel (1940) reported that testosterone propionate administered orally with bile salts was considerably more active in controlling the vasomotor symptoms of the menopause in women than was testosterone propionate alone. Hence these authors concluded that bile salts facilitate the absorption of testosterone propionate from the gastro-intestinal tract. Wells, Burrill and Greene (1940), on the other hand, were unable to show that the administration of desoxycholic acid increased the effectiveness of orally administered testosterone propionate in stimulating the prostate and seminal vesicles of the castrated rat. Selye (1943) also concluded that bile plays no important role in the enteral absorption of steroid hormones since the intragastric administration of desoxycorticosterone acetate, progesterone or testosterone to rats, in which the common bile duct had been ligated, produced a state of anaesthesia.

In the present experiments the role of bile in the absorption of steroids was assessed by determining whether the metabolites of dehydroisoandrosterone, progesterone and desoxycorticosterone appear in the urine, following the intragastric administration of these substances to rabbits in which bile was excluded from the gastro-intestinal tract by transection of the common bile duct.

Received for publication February 24, 1948.

[1] This investigation was supported in part by a grant from the Charles E. Frosst and Company, Montreat, to the McGill University Clinic.

The authors are greatly indebted to Dr. E. Schwenk of the Schering Corporation for supplying the progesterone and desoxycorticosterone acetate used in these studies, and to Dr. G. Holden of the Charles E. Frosst and Company for generously providing dehydroisoandrosterone.

[2] Present address, University of Montreal, Montreal.

METHODS

Each of the steroids studied was administered to six adult rabbits which were divided into groups of three. One group of unoperated animals served as a control and the other group consisted of rabbits in which the common bile duct had been cut between two ligatures four days prior to the administration of the steroid. In each instance the total dose of steroid was dissolved in 5 ml. of a 10% solution of ethyl alchol in propylene glycol and administered by stomach tube. Following the administration of the steroid the animals were placed in metabolism cages and the urine collected separately from each animal at twenty-four hour intervals.

Metabolism of dehydroisoandrosterone: Since preliminary studies revealed that the 17-ketosteroids excreted by the rabbit after the administration of

TABLE 1. EFFECT OF CONCENTRATION OF HYDROCHLORIC ACID AND DURATION OF BOILING ON LIBERATION OF 17-KETOSTEROIDS FROM URINE OF RABBITS TREATED WITH DEHYDROISOANDROSTERONE

Volume of HCl/100 ml. of urine	Duration of boiling	17-ketosteroids/100 ml. of urine
ml.	min.	mg.
2	15	2.05
2	30	2.52
5	15	3.65
5	30	4.5
5	60	5.2
10	15	5.15
10	30	5.05
10	60	5.8
15	15	5.8
15	30	5.5
15	60	5.3
20	15	5.7
20	30	5.5

dehydroisoandrosterone are in the conjugated form, it became necessary to establish the optimum conditions for the hydrolysis and extraction of these substances. To this end a total of 700 mg. of dehydroisoandrosterone were administered by gavage to three adult male rabbits whose urine was collected for four days. The urine was pooled and adjusted to pH7. Aliquots of 100 ml. of this urine were brought to the boiling point and different amounts (2 to 20 ml.) of concentrated hydrochloric acid were added and boiling continued for periods ranging from 15 to 60 minutes as shown in Table 1. Following rapid cooling the urine was extracted with ether; the neutral ether-soluble fraction was prepared in the usual manner and the kentonic steroids separated from the non-ketonic material by treatment with Girards' reagent T. Determination of the amounts of 17-ketosteroids present in the ketonic fraction was made be means of the Holtorff and Koch (1940) modification of the Zimmerman color reaction. As can be seen from Table 1 the maximum yield of 5.8 mg. of 17-ketosteroids was obtained either by the addition of 10 ml. of hydrochloric acid followed by boiling for 60 minutes, or by the addition of 15 ml. of hydrochloric acid with boiling for 15 minutes. The latter conditions were selected for the experiments herein described.

Each male rabbit received a single dose of 200 mg. of dehydroisoandrosterone. Urine was collected with hydrochloric acid as a preservative at twenty-four hour intervals for three days. The amount of 17-ketosteroids excreted in each twenty-four hour period was determined by the method described above.

Metabolism of progesterone and desoxycorticosterone acetate: The progesterone treated female animals each received 175 mg. of the steroid. Urine was collected under a layer of 75 ml. of butyl alchol containing 0.1 ml. of cresol, daily, for a period of 6 days. Each twenty-four hour collection of urine was extracted with butyl alchol; the butanol extracts were combined and the amount of sodium pregnanediol glucuronide present determined gravimetrically by the method of Venning (1938).

The female rabbits treated with desoxycorticosterone acetate each received 100 mg. of the steroid, The urine was collected and processed as described above for progesterone.

The identity of the precipitate isolated by the Venning procedure from the urine of the progesterone and desoxycorticosterone treated animals was established by melting point and by the isolation of pregnanediol-3 (α), 20, (α) from the products of acid hydrolysis.

RESULTS AND DISCUSSION

The rabbit, whose urine normally contains little or no 17-ketosteroids, excretes these substances following the administration of dehydroisoandrosterone. Previous studies (Hoffman and Desbarats, 1946) have shown that $\Delta^{3,5}$-androstadienone-17, dehydroisoandrosterone and etiocholanol-3 (α), one-17 are the 17-ketosteroids excreted by the rabbit treated with dehydroisoandrosterone. As shown in Table 2, the normal animals which received 200 mg. of dehydroisoandrosterone by gavage excreted from 38 to 44.3 mg. of 17-ketosteroids. The major portion appeared in the twenty-four period following the administration of the steroid. A smaller amount was excreted during the second day and by the third day the urine was practically free of 17-ketosteroids. Similarly the rabbits with transected bile ducts excreted 17-ketosteroids following the administration of dehydroisoandrosterone. The amount excreted varied from 34.1 to 54.9 mg. The completeness of the extra-hepatic biliary obstruction in these animals was established by the rapid development of jaundice and the occurrence of death within 8 to 35 days following the operation; at autopsy no accessory bile ducts could be demonstrated. Since these animals excreted 17-ketosteroids following the administration of dehydroisoandrosterone, it must be concluded that this substance was absorbed from the gastro-intestinal tract in the absence of bile.

Four days were allowed to elsape after transection of the bile duct before administering the steroid in order to exclude the presence of bile from the upper gastro-intestinal tract at the time of treatment. To obviate the possibility that even after this period a small amount of bile might still be present, the experiment described above was repeated on animals in which the bile duct had been severed at least fifteen days before treatment. As before 17-ketosteroids appeared in

TABLE 2. EXCRETION OF 17-KETOSTEROIDS FOLLOWING ADMINISTRATION OF DEHYDROISOANDROSTERONE BY GAVAGE TO NORMAL RABBITS AND RABBITS WITH TRANSECTED BILE DUCTS

Rabbit no.	Condition of animal	Dehydroisoandrosterone administered	Amounts of 17-ketosteroids excreted			
			Day 1	Day 2	Day 3	Total
		mg.	mg.	mg.	mg.	mg.
89	Normal control	200	35.1	6.82	0.57	42.49
91	Normal control	200	33.1	3.89	1.01	38.0
92	Normal control	200	36.24	6.02	2.11	44.37
83	Transected bile duct	200	45.6	5.37	1.35	52.32
86	Transected bile duct	200	36.12	17.6	1.24	54.96
88	Transected bile duct	200	16.4	15.74	2.02	34.16

the urine following the administration of dehydroisoandrosterone.

The rabbit is known to convert exogenous progesterone and desoxycorticosterone acetate to pregnanediol which is excreted in the urine as the glucuronide (Heard, Bauld and Hoffman, 1941; Hoffman, Kazmin and Browne, 1943). In the present experiments, which are summarized in Table 3, the administration of 175 mg. of progesterone to normal female animals was followed by the excretion of 39.2 to 69.8 mg. of sodium pregnanediol glucuronide in the urine. Similarly the animals with transected bile ducts excreted 47.9 to 129.4 mg. of pregnanediol glucuronide after progesterone treatment. In these latter animals the identity of the precipitate was established by its physical properties and by the isolation of pregnanediol -3 (α), 20 (α) from the products of its acid hydrolysis. Here as in the case of the dehydroisoandrosterone experiments, the completeness of the interruption of the extra-hepatic biliary tract, was established by the clinical course and by the autopsy findings.

As shown in Table 4, similar results were obtained following treatment with desoxycorticosterone acetate. From 20.3 to 35.3 mg. of sodium pregnanediol glucuronide appeared in the urine of the normal animals given 100 mg. of desoxycorticosterone acetate by gavage. The animals with transected bile ducts treated the same way excreted 28 to 31.5 mg. of the pregnanediol conjugate which was identified in the manner described above.

TABLE 3. EXCRETION OF PREGNANEDIOL GLUCURONIDE FOLLOWING ADMINISTRATION OF PROGESTERONE BY GAVAGE TO NORMAL RABBITS AND RABBITS WITH TRANSECTED BILE DUCTS

Rabbit no.	Condition of animal	Progesterone administered	Pregnanediol glucuronide excreted
		mg.	mg.
79	Normal control	175	69.8
80	Normal control	175	39.2
81	Normal control	175	40.8
78	Transected bile duct	175	47.9
104	Transected bile duct	175	129.4
124	Transected bile duct	175	103.2

To exclude the possibility of the presence of residual bile in the gastro-intestinal tract of the treated animals, progesterone and desoxycorticosterone acetate were administered separately to rabbits in which bile duct transection had been performed at least fifteen days prior to treatment. Under these conditions pregnanediol was still excreted in the urine. Since pregnanediol appears in the urine following the intragastric administration of these two substances to animals in which bile is absent from the gastro-intestinal tract, it may be concluded that bile salts are not essential for their absorption.

TABLE 4. EXCRETION OF PREGNANEDIOL GLUCURONIDE FOLLOWING ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE BY GAVAGE TO NORMAL RABBITS AND RABBITS WITH TRANSECTED BILE DUCTS

Rabbit no.	Condition of animal	DCA administered	Pregnanediol glucuronide excreted
		mg.	mg.
118	Normal control	100	26.8
119	Normal control	100	35.3
121	Normal control	100	20.3
118	Transected bile duct	100	31.5
121	Transected bile duct	100	30.2
122	Transected bile duct	100	28.0

Although the amounts of metabolites excreted by the animals with transected bile ducts were as great or greater than the amounts excreted by the control animals, it cannot be concluded that the absorption of the steroids studied was as good in the experimental animals as in the control animals. At least two other factors may have played a role in determining the amounts of the administered substances which appear as metabolites in the urine. First, it has been observed that bile serves as a medium of excretion for the metabolites of steroid hormones. The administration of α-estradiol and testosterone to the human subject and the dog is followed by excretion in the bile of part of the metabolites of these substances (Cantarow, Rakoff, Paschkis, Hansen and Walkling, 1942, a.b.; Cantarow, Paschkis, Rakoff and Hansen, 1943; Paschkis, Cantarow, Rakoff, Hansen and Walkling, 1944). Therefore transection of the common bile duct, as was practiced in the present experiments, by interfering with a possible channel of excretion might have had some influence on the amounts of the metabolites excreted in the urine. Secondly, there is considerable evidence to indicate that the liver plays an important role in the metabolism of steroid hormones. In the rabbit, for example, it has been shown that the liver is capable of inactivating exogenous estrone (Biskind and Meyer, 1943) and progesterone (Masson and Hoffman, 1945; Engel, 1946). This is a possible factor in the present experiments since it is likely that the biliary obstruction which was produced would have caused liver damage, which in turn might have affected the metabolism of the administered steroids and thus indirectly have influenced the amounts of metabolites appearing in the urine.

Inspection of the results herein reported suggest that in the case of the progesterone-treated rabbits the amounts of pregnanediol in the urine of the animals with transected bile ducts were actually greater than in the urine of the control animals. This could be explained as a consequence of either or both of the two factors discussed above. Experiments are now in progress to determine the effect of liver damage, produced by prolonged extra-hepatic biliary obstruction, on the metabolism of steroid hormones and related substances.

SUMMARY

Crystalline dehydroisoandrosterone, progesterone and desoxycorticosterone acetate were administered by gavage to normal rabbits and rabbits in which bile was prevented from reaching the gastro-intestinal tract by transection of the common bile duct. In each group of animals the amounts of metabolites of the administered steroids, excreted in the urine, were determined. It was found that the animals with transected bile ducts, treated with dehydroisoandrosterone, excreted as much 17-ketosteroids as did the normal animals similarly treated. The rabbits with biliary obstruction which were given progesterone and desoxycorticosterone acetate excreted pregnanediol glucuronide in amounts equal to or greater than control animals treated in the same way. It was concluded, therefore, that dehydroisoandrosterone, progesterone and desoxycorticosterone acetate can be absorbed from the gastro-intestinal tract of the rabbit in the absence of bile.

ACKNOWLEDGMENT

The authors wish to thank Dr. J. S. L. Browne for his interest and help in this study.

REFERENCES

Biskind, G. R., and M. A. Meyer: *Proc. Soc. Exper. Biol. & Med.* 53: 91. 1943.
Cantarow, A., K. E. Paschkis, A. E. Rakoff and L. P. Hansen: *Endocrinology* 33: 309. 1943.
Cantarow, A., A. E. Rakoff, K. E. Paschkis, L. P. Hansen and A. A. Walkling: *Endocrinology* 31: 515. 1942.
Cantarow, A., A. E. Rakoff, K. E. Paschkis, L. P. Hansen and A. A. Walkling: *Proc. Soc. Exper. Biol. & Med.* 49: 707. 1942.
Engel, P.: *Endocrinology* 38: 215. 1946.
Heard, R. D. H., W. S. Bauld and M. M. Hoffman: *J. Biol. Chem.* 141: 709. 1941.
Hoffman, M. M., and M. L. Desbarats: *Federation Proc.* 5: 49. 1946.
Hoffman, M. M., V. E. Kazmin and J. S. L. Browne: *J. Biol. Chem.* 147: 259. 1943.
Holtorff, A. F., and F. C. Koch: *J. Biol. Chem.* 135: 377. 1940.
Masson, G., and M. M. Hoffman: *Endocrinology* 37: 111. 1945.
Paschkis, K. E., A. Cantarow, A. E. Rakoff, L. P. Hansen and A. A. Walkling: *Proc. Soc. Exper. Biol. & Med.* 55: 127. 1944.
Schonheimer, R.: *Biochem. Ztschr.* 147: 258. 1924.
Selye, H.: *Endocrinology* 32: 279. 1943.
Silberman, D., H. M. Radman and A. R. Abarbanel: *Am. J. Obst. & Gynec.* 39: 332. 1940.
Venning, E. H.: *J. Biol. Chem.* 126: 595. 1938.
Wells, J. A., M. W. Burrill and R. R. Greene: *Proc. Soc. Exper. Biol. & Med.* 45: 452. 1940.

LYMPHOCYTE DISCHARGE FROM THE ISOLATED RABBIT SPLEEN BY ADRENAL CORTICAL EXTRACT

OSCAR HECHTER

From the Worcester Foundation for Experimental Biology, Shrewsbury, Mass., and the Department of Physiology, Tufts Medical School

BOSTON, MASS.

IN AN ATTEMPT to elucidate the mechanism whereby adrenal cortical hormones (ACH) increase the rate of lymphocyte dissolution within lymphoid organs (White and Dougherty, 1946), studies were undertaken to investigate the effect of ACH upon isolated lymphoid organs, perfused with whole blood. For the first studies, the effect of adrenal cortical extract (ACE) upon the isolated spleen was undertaken on the basis that if ACE acted directly upon lymphoid tissue to accelerate lymphocytic dissolution, this would be evidenced by a significant decrease in the number of circulating lymphocytes in the perfusion blood and by histological evidence of lymphocyte dissolution in the perfused spleen. When the first experiments were performed, contrary to expectations, it was observed that addition of ACE to the perfused spleen system, induced a prompt significant rise in the number of circulating lymphocytes, without affecting the number of circulating erythrocytes or polymorphonuclear leucocytes. This unexpected response of the isolated spleen to ACE was therefore investigated in detail.

The purpose of this paper is to present the results of these studies. The data indicate that the *in vitro* splenic response to ACE is specific in that ACE does not produce a similar effect upon either the perfused thymus, liver, or lung; and that perfusing the isolated spleen, with certain doses of estradiol dipropionate, desoxycorticosterone acetate, epinephrine, or sugar does not lead to splenic lymphocyte discharge.

METHODS

General: Rabbits of various strains, weighing between 3 to 4 kg. furnished the blood and organs used in the perfusion experiments. The isolated organs studied were spleen, thymus, liver and lung. All organs were perfused with whole heparinized blood which had previously been circulated through lung for at least 60 minutes to remove vasoconstrictor substances and possible emboli or foreign particles from the perfusion medium.[1] Spleen or thymus,

Received for publication February 24, 1948.

Aided in part by a grant from the G. D. Searle and Company.

[1] It has been observed in the course of perfusing various rabbit organs (e.g. adrenals, ovary, testicle, kidney, etc.) with heparinized whole blood at a mean arterial pressure of 100 mm. Hg., which has not been passed through lung, that the organ perfusion is unsatisfactory. Thus, the initial blood flow through the organ is subnormal; as perfu-

unless otherwise stated, was perfused with a regulated pulsatile pressure (pulse rate 72 per minute) so that the mean arterial pressure near the tip of the cannula was maintained at a constant level (100 ± 10 mm. Hg.) Liver and lung were perfused at a non-pulsatile pressure equivalent to approximately 20 mm. Hg.

At varying time intervals, before and after the addition of the agent under investigation to the circulating perfusion medium, blood samples were withdrawn from the "arterial side" of the pump for analysis of RBC, WBC, differential white cell count and glucose (determined on Folin-Wu filtrates by a modification of the method of Schales and Schales 1945). Counting of RBC and WBC was performed, in duplicate, in the usual manner; differential analysis of leucocytes was made by counting 100 cells on air dried smears stained with Wrights' stain.

While the perfusion apparatus used in this study was designed to permit sterile perfusion of organs, it was not considered practical to attempt daily sterile perfusion experiments. To limit the influence of bacterial infection without utilizing tedious sterile perfusion technics (*cf.* Carrell and Lindbergh, 1938) penicillin and streptomycin were added to the perfusion medium. This combination of antibiotics effectively prevented the development of those types of bacterial growth in the perfusion system which otherwise contaminate it, and permitted short-term perfusion experiments virtually uncomplicated by the factor of bacterial infection.[2]

---

sion is continued the blood flow tends to decrease still further, and the organs become hemorrhagic, cyanotic, and edematous. These difficulties are largely avoided by passing blood through lung prior to using the blood for perfusion studies. The role of the lung in facilitating perfusion of isolated organs has usually been ascribed to removal of vasoconstrictor substances liberated in blood (cf. Hemingway, 1931), and to the retention of foreign particles or small emboli within the lung. However, it should be pointed out that the viscosity of blood is significantly reduced as the result of preliminary lung perfusion and it is possible that this lowering of blood viscosity is likewise important for the successful perfusion of isolated organs with whole blood. It may be mentioned that if serum is used as the perfusion medium instead of whole blood, it has been found that the preliminary lung perfusion is not necessary for successful perfusion of organs.

[2] Using a clean but not sterile technic for removal of blood and organs, and a non-sterile pump, for perfusion of lung, then spleen, it has been observed that without penicillin and streptomycin, after 5–6 hours of perfusion that 0.1 cc. of the perfusion medium placed on a nutritive agar plate and then streaked, gives rise to several hundred to a thousand colonies upon incubation at 37° C. for 48 hours. (Usually *staph aureus, staph albus, B. Coli.*) In six similar experiments wherein 2–5 Oxford units penicillin and 300–500 μg. streptomycin SO_4 per ml. blood was added to the pump, it was observed that 0.1 cc. blood removed after 5–6 hours of perfusion gave rise to 0, 0, 2, 4, 6, 6, and 10 colonies (mainly yeasts and less frequently fungi) upon agar plates incubated for 48 hours at 37° C. It may be mentioned in passing that these isolated colonies of yeasts and fungi did not grow upon the streaks, but only where the blood sample was dropped on the agar plate. The differences in perfusion experiments obtained with antibiotics are more striking with longer periods of perfusion. Thus, without antibiotics, blood became completely hemolyzed after 12 to 16 hours of perfusion and methemoglobin is formed. With penicillin and streptomycin it has been found in experiments with perfused adrenal glands that the majority of erythrocytes are still intact after 24 hours despite the presence of a yeast infection, and that the perfused organs showed only slight evidence of damage as revealed by histological examination. It may be mentioned in passing that if a factor effective against yeasts and fungi, but non-toxic to mammalian tissues, were available, combination of this agent with penicillin and streptomycin might permit sterile perfusion of organs without resorting to tedious aseptic and antiseptic technics.

Perfusion Apparatus: The perfusion apparatus illustrated in Fig. 1 was designed to permit the pulsatile perfusion of organs with whole blood for prolonged periods of time. Essentially it consists of two separate circuits, one for non-pulsatile perfusion of the lung to prepare the blood for subsequent perfusion; the other for pulsatile-perfusion of organs.[3] The apparatus consists of an organ chamber for pulsatile perfusion *A;* a blood reservoir *B;*

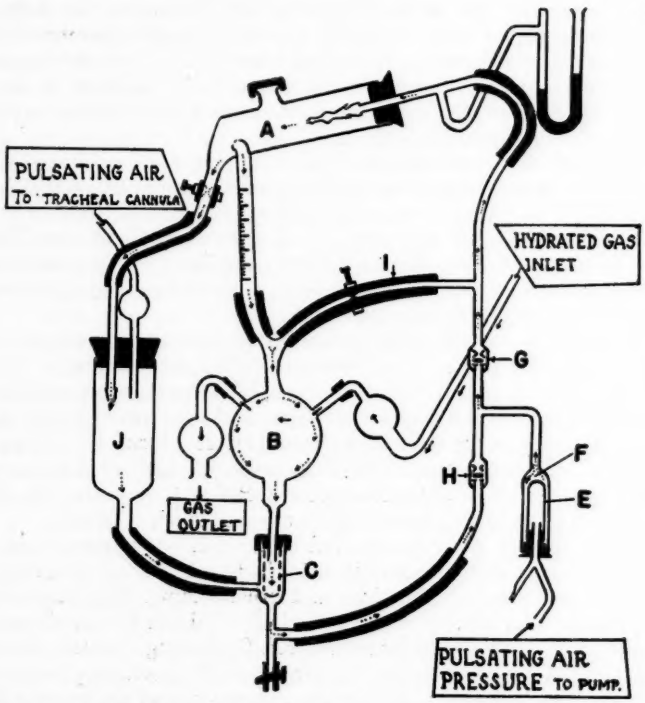


FIG. 1. A schematic drawing of the perfusion apparatus. The broken arrows show the course of blood circulation through the pump; the solid arrows show the course of oxygen through the apparatus.

a blood filter *C;* a pump, consisting of a rubber finger cot *E;* which is inserted into a pump reservoir F and is interposed between two one-way glass valves *G* and *H;* a pulse pressure regulator *I;* an organ chamber used for non-pulsatile perfusion *J;* and glass and other tubing which connects the separate parts of the apparatus. All glass in the apparatus is pyrex; the tubing used to connect the various parts of the apparatus may be either rubber

[3] When serum, artificial media not containing cells, or highly diluted whole blood is used as the perfusion medium, preliminary perfusion of the blood through lung is not necessary. Accordingly, when these media are used for perfusion studies, the circuit for lung perfusion is not included; the apparatus is then more compact and sterilization is simpler. The shape or size of the organ chambers *A* and *J* may be modified depending upon the organ to be studied.

(Fenwal) with or without an inside coating of dimethyl silicone,[4] or Tygon flexible tubing. The apparatus is mounted in a constant temperature room maintained at 38° C., saturated with glycol vapor to sterilize the air.

The apparatus is actuated by means of compressed air (from a storage tank containing 20 lbs. per sq. in.) which is passed through a rotating valve (similar to that described by Lindbergh, 1935), thus creating a pulsating air pressure. The pulsating air is directed into the finger cot *E* of the pump, which then pulsates. As the finger cot expands, blood from the pump reservoir *F* is pumped past valve *G* into the line toward organ chamber *A*; on the off beat the finger cot contracts, sucking blood into *F* from the blood reservoir *B*, through filter *C*. Upon arriving at the organ chamber *A*, the blood can take either of two alternative routes depending upon whether a pulsatile or non-pulsatile perfusion is desired.

For non-pulsatile perfusions of lung and liver using pressures approximately 20 mm. Hg., the organ chamber *A* is used as an overflow vessel placed approximately 40 cm. above the cannula of the perfused liver or lungs in organ chamber *J*. This is achieved by having two outlets in organ chamber *A*—the lower outlet connecting with the cannula of the perfused lung or liver, while the other outlet takes the blood pumped in excess of liver or lung blood flow back to the blood reservoir *B*.

For a pulsatile perfusion the non-pulsatile perfusion circuit is clamped off; the organ to be perfused with pulsatile pressure is inserted in Chamber *A*, and the cannula is attached to the line. Blood pumped upwards from *F* then enters the nutrient artery of the organ, and then after passing through the organ runs down the floor of the chamber. The blood then flows down the sides of a calibrated tube connecting the organ chamber *A* and reservoir *B*, and thus passes into the blood reservoir *B*. Before the blood is recirculated it passes through a nylon stocking filter of small mesh (Fenwal).

Oxygenation of the blood is achieved by the following arrangement: The blood leaving organ chamber *A* returns to the blood reservoir as a film which adheres to the surface of the glass and other tubing. Upon entering the spherical blood reservoir, the blood spreads as a thinner film, and then flows down to mix with the blood in the reservoir *B*. By passing a continuous stream of 95% O_2—5% CO_2 gas mixture hydrated at the perfusion temperature, through the blood reservior *B*, effective oxygenation of the moving film of blood is achieved without vaporation. Foaming of the blood during oxygenation is prevented by avoiding the passage of a gas mixture directly through the blood, and ensuring that the perfusion medium always flows on a surface. In this latter connection, it is important that the tubing connecting organ chamber *A* with the blood reservoir should have a minimal internal diameter of 10 mm.

The pump is interposed between two one-way valves *G* and *H*, and consists of a glass reservoir (length 10 cm. and 2.5 cm. I. D.), into which a finger cot is fitted by means of a rubber stopper. The maximal output of the pump is dependent upon the pulsation rate and the volume of the pump reservoir capable of being contracted by expansion of the rubber finger cot. The vol-

[4] Rubber tubing can be coated with silicone polymers which then form a non-wettable surface which delays blood clotting (Jacques *et al.*, 1947). By coating all surfaces of a perfusion pump with silicone, it has been possible to perfuse liver with whole blood without the addition of any anticoagulants to prevent clotting in the system (Roche e Silva *et al.* 1947).

ume of the pump reservoir also determines to an important degree the minimal amount of blood necessary to fill the apparatus. The one-way valves (obtained from Macalaster Bicknell Company, Cambridge, Mass.) illustrated in Fig. 1 require special mention. When properly made, these valves do not stick, nor do blood cells settle out at the seat of the valve. They consist of a circular glass disc which sits upon a platform of glass at the end of the glass tubing; the surfaces of the disc as well as the platform are polished, not ground, thus minimizing the possibility of red cell rupture in opening and closing of the valve.

Mean arterial pressure measurement during organ perfusion was obtained with a damped Hg. manometer inserted into the line at a point just before the blood enters the organ chamber *A* and the nutrient artery of the organ (*cf.* Fig. 1). By using calibrated cannulae wherein the drop in pressure between this point in the line and the artery of an organ has been previously measured, the *mean* pressure in the artery of a perfused organ can be ascertained from the Hg. manometer.

Arterial pulse-pressure was not controlled during these perfusion experiments; however, visual examination of the artery of the organ during the course of perfusion afforded a gross qualitative index of pulse pressure. Where control of pulse-pressure is desired, the arrangement illustrated in Fig. 1, where a portion of the pump output may be short-circuited, provides a method of obtaining varying pulse pressure at a particular mean pressure. The amount of blood which is short-circuited is regulated by means of a Hoffman clamp.

Procedure for perfusion: A total of 110 to 150 cc. of blood, collected by heart puncture from two heparinized rabbits, was added to a flask containing 20 mg. heparin (Upjohn), 5000 Oxford U. penicillin sodium and 50 mg. streptomycin sulfate in a total volume of 3 cc. The contents of the flask were mixed and added to the perfusion pump. Immediately thereafter, the lungs and trachae of one of the exsanguinated rabbits were removed and cannulae were introduced into the pulmonary artery and trachea. The lungs were first flushed with Tyrode solution, introduced into the non-pulsatile organ chamber *J*, and were then perfused with the previously collected blood, for at least 60 minutes. During this period the lungs were inflated and deflated by a pulsating air current directed into the tracheal cannula. The average blood flow through rabbit lungs perfused under these conditions varied between 60 and 150 cc. per minute.

While the lung perfusion was proceeding, a third rabbit was prepared to furnish the organ for perfusion. The animal was anesthetized with pentobarbital and then injected intravenously with 10 mg. of heparin. The necessary blood vessel ligations were made; then the organ was removed, cannulated and flushed with Tyrode solution. After the lungs were removed from the apparatus, the organ to be perfused was inserted into the appropriate chamber. Thus, another lung or liver was placed in the non-pulsatile chamber *J* replacing the previously perfused lung; thymus or spleen was placed in the pulsatile organ Chamber *A*, and the non-pulsatile perfusion circuit was cut off by a clamp.

Spleen was prepared for perfusion by inserting a cannula into the abdominal aorta above the coeliac artery, ligating the aorta below the coeliac artery. In all splenic preparations the gastrohepatic artery was tied off; in some prep-

arations, all of the gastric and pancreatic branches of the lieno-gastric artery and vein were ligated; in others, the pancreatic and some of the gastric branches of the splenic artery were left open as bleeders. With both types of splenic preparations, it was noted that in approximately 25 per cent of the preparations, the circulation through the spleen rapidly became blocked, as evidenced by the cyanotic appearance of the organ and (where this was possible) by measurements of blood flow. These ineffectively circulated splenic preparations were discarded and are not included in the results to be reported here. In most splenic preparations, a variable amount of hemorrhage into the connective tissue surrounding the blood vessel occurs; however, this did not appear to significantly affect the circulation through the organ, as evidenced by coloration of the perfused spleen.

Thymus was prepared for perfusion by inserting a cannula into the descending portion of the thoracic aorta directed toward the aortic arch, ligating the ascending aorta, the subclavian arteries behind the internal mammary arteries, and the common carotid arteries above the superior thyroid arteries.

Lungs and liver were perfused in the ordinary fashion: liver via the portal vein, lung via the pulmonary artery.

In all instances, care was taken to ensure that no air emboli were present either in the cannula or the blood vessels of the excised organ. Furthermore it should be mentioned that in all of the perfused preparations the venous out-flow was not cannulated, but allowed to issue from the opened veins. Upon conclusion of the experiment, the perfused tissue was fixed in 10 per cent formalin for subsequent histological examination.

Sampling and addition of agents: "Arterial blood" samples were obtained with a hypodermic syringe by inserting the needle into a rubber connection at a point in the line just before the blood entered the nutrient artery of the organ. "Venous samples," when taken, were obtained from the outflow issuing from the organ by clamping the rubber tubing connecting the organ chamber *A* with the blood reservoir *B* and using a syringe and needle for sampling. Blood flow through the organ was measured (in those cases where all arterial bleeders were ligated) by clamping the same tubing, and measuring the time (in 0.1 second) necessary for the calibrated tube to fill to a particular volume.

Addition of various agents (sugar, hormones, etc) was made by injecting solutions through the rubber cap in the organ chamber *A: thus in these experiments all injected materials entered the venous side of pump,* and were filtered before entering the perfused organ. Water soluble agents added in this manner readily mix with the circulating blood. When agents dissolved in oil vehicles are added, the following course of events occurs: The blood issuing from the organ washes the oil solution into the blood reservoir *B* where, for the most part, the oil remains on the surface of the blood in the reservoir. Most of those oil globules which pass down the reservoir with the circulating blood, are trapped by the nylon filter and are prevented from entering the circulation.

Agents investigated: Two ACE preparations were studied, a lipo-adrenal cortex extract (Upjohn) and an aqueous extract (prepared by Endo Products Co.). The lipo-adrenal cortex extract henceforth referred to as ACE (oil) is dissolved in a cottonseed oil vehicle containing 1 mg. per cc. chlorobutanol

as preservative. The aqueous extract (1 cc. equivalent to 60 gms. fresh tissue) henceforth referred to as ACE (aq.) is dissolved in 0.9 per cent NaCl and contains 1:10,000 phenyl mercuric salicylate. In addition to these ACE preparations, desoxycorticosterone acetate and estradiol dipropionate (both in a peanut oil vehicle) epinephrine hydrochloride (Parke-Davis) and glucose were tested to determine their influence upon splenic discharge of lymphocytes.

RESULTS

Before starting to examine the effect of ACE upon the perfused spleen, it was necessary to obtain information on three preliminary points concerning the formed elements of blood: (a) the effect of circulating the blood, with and without added ACE, through the apparatus in the absence of any organ; (b) the effect of preliminary perfusion of blood through the lung; and (c) the effect of spleen perfusion in the absence of added ACE.

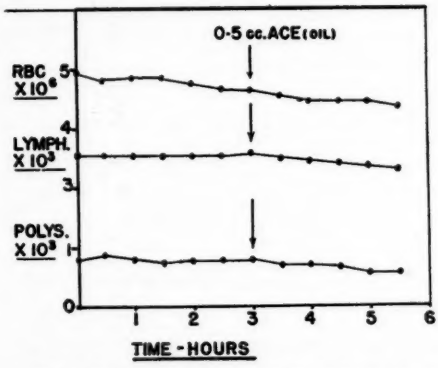


FIG. 2. The effect of circulating blood through the apparatus, upon the formed elements of blood, in the absence of an organ, before and after the addition of ACE. All values in terms of cells per mm.³.

The Effect of Circulating Blood through the Apparatus, with and without ACE. To determine the effect of the *in vitro* conditions of circulation upon the cellular elements of blood, whole blood was circulated through the apparatus in the absence of any organ in four experiments. In three of these experiments ACE (oil) was added to the apparatus after the blood had been circulated for several hours to test the possibility that ACE might directly affect the number of circulating red or white blood cells. Fig. 2 illustrates the results of a typical experiment of this type.

In this experiment the volume of the perfusion medium was 155 cc.; blood was pumped through a cannula at a rate of 180 cc. per minute; 24 mg. glucose was added every 30 minutes to maintain the blood sugar at a level of approximately 130 mg. per cent. The blood was circulated for three hours without ACE, and then for an additional 2.5

hours after the addition of 0.5 cc ACE (oil). It will be seen that the number of circulating erythrocytes, lymphocytes and polynuclear leucocytes in the blood circulated through the system without an organ remain essentially unchanged, during the duration of the experiment.

In the other experiments, similar results were obtained as regards the minimal destruction or removal of erythrocytes and leucocytes in the course of recirculating blood through the apparatus *per se* with or without added ACE (oil) when the rate of flow through the apparatus was varied from 20 to 200 cc. per minute, or when the blood sugar level was permitted to fall without replenishment with sugar.

These results demonstrate that circulation of blood through the perfusion apparatus *per se* produces only minimal changes in the

TABLE 1. EFFECT OF PERFUSING BLOOD THROUGH LUNG UPON CELLULAR ELEMENTS AND SUGAR CONTENT OF THE BLOOD

Exp. No.	Blood vol.	Av. blood flow	Initial				% Decrease as result of lung perfusion							
							30 min.				60 min.			
			RBC*	L†	P‡	G§	RBC	L	P	G	RBC	L	P	G
	cc.	cc./min.												
1	120	110	5.60	9.88	5.25	98	11	49	79	16	16	53	90	30
2	120	60	5.40	5.57	2.30	101	15	21	48	14	17	22	63	32
3	138	—	5.03	4.10	1.20	105	3	15	52	15	8	36	75	29
4	110	90	4.83	4.75	2.10	93	5	47	78	12	30	53	96	24
5	123	60	4.97	2.98	1.28	99	2	18	48	18	5	65	92	32
6	125	85	4.36	3.52	1.20	—	0	8	76	—	3	20	85	—
Mean							6	26	62	15	13	41	83	29

* RBC ($\times 10^6$) per mm.^{3}.
† L is the absolute lymphocyte count ($\times 10^3$) per mm.^{3}.
‡ P is the absolute polymorphonuclear leucocyte count ($\times 10^3$) per mm.^{3}.
§ G is the blood glucose in mg. %.

number of circulating erythrocytes and lymphocytes, and that under these conditions ACE does not influence the number of circulating blood cells.

The Effect of Preliminary Lung Perfusion upon the Cellular Elements of Blood. Table 1 illustrates the changes in the formed elements of blood produced as a result of preliminary lung perfusion. It will be seen that as a result of passage of blood through the lungs for 60 minutes that the RBC count is reduced an average of 13 per cent, lymphocytes 41 per cent, and polymorphnuclear leucocytes 83 per cent; thus the leucocytes present in the blood after perfusion of lung are mainly lymphocytes.

Visual inspection of the lung reveals that certain areas become hemorrhagic and edematous during the course of perfusion; histological examination of these areas reveals endothelial ruptures with passage of mainly erythrocytes and a few leucocytes into the alveolar air spaces. It would therefore appear that the disappearance of a portion of the red and white blood cells during lung perfusion could be accounted for on the basic of this mechanism.

Effect of Perfusing Spleen in the Absence of Added ACE. Table 2 shows

the change in cellular components of blood produced as a result of pulsatile perfusion of spleen with whole heparinized blood (previously perfused through lung) at a constant mean arterial pressure of 100 ± 10 mm. Hg. It will be seen that the number of circulating erythrocytes uniformly decrease at a slow rate (an average decrease of 2 per cent per hour), but that the lymphocyte count remains, on the average, relatively constant. Similar results were obtained whether glucose was or was not added to the perfusion system to maintain the initial glucose concentration.

Histological examination of spleens perfused for five hours as compared to non-perfused spleen revealed significant depletion of lymphocytes from the splenic red pulp, with partial replacement by agglutinated and conglutinated RBC and hemosiderin. The number and size of the Malpighian corpuscles and lymphoid follicles appeared

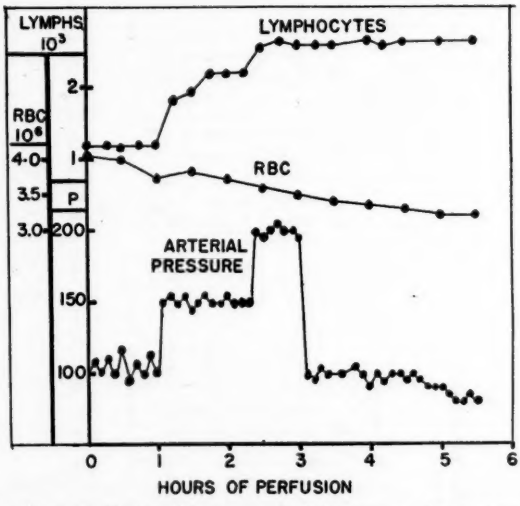


FIG. 3. The effect of altering the mean arterial pressure of a perfused spleen, upon the number of circulating lymphocytes and erythrocytes. All values in terms of cells per mm.³. Arterial pressure in mm Hg.

somewhat smaller, although the signifiance of this change was diminished by the variability observed between different perfused splenic preparations. Further, the macrophages of the perfused spleens contained large amounts of debris, possibly of nuclear origin. These findings demonstrating loss of lymphocytes from the perfused spleens taken together with the fact that the number of lymphocytes in the circulation remains relatively constant suggests that the perfused spleen destroys circulating lymphocytes on one hand and adds splenic lymphocytes to the circulation on the other hand.

In the next experiments the effect of increasing splenic perfusion pressure upon the circulating blood cells was determined. In four ex-

TABLE 2. THE EFFECT OF SPLEEN PERFUSION, IN THE ABSENCE OF ADDED ACH, UPON THE CELLULAR ELEMENTS OF BLOOD

Exp. no.*	Blood sugar maintained§	Initial		Percentage change as result of spleen perfusion													
				30 min.		60 min.		90 min.		120 min.		180 min.		240 min.		300 min.	
		R†	L‡	R	L	R	L	R	L	R	L	R	L	R	L	R	L
1	No	4.00	1.90	−3.5	− 4.2	−5.5	−8.9	−7.7	−12.6	−9.0	−16.8	−10.0	−19.0	−14.0	−19.0	−16.0	−19.0
2	No	4.06	3.92	−1	− 5.4	−4.2	−9.3	−7.6	−15.8	−8.8	−15.5	− 9.6	−11.2	−14.1	−10.5	−18.8	−10.0
3	No	4.01	1.77	—	—	−3.7	+1.7	—	—	−7.2	+ 7.3	− 7.5	+ 4.5	−10.2	+13.0	−12.0	+11.0
4	Yes	4.32	1.35	−3.5	−10.4	−7.0	0	−7.2	− 3.8	−7.3	− 2.2	− 8.3	− 5.2	−10.0	− 7.4	−11.0	− 6.7
5	Yes	4.81	4.30	−1.6	− 1.4	−0.8	−1.4	−1.2	− 2.3	−3.1	− 2.1	− 2.5	− 8.0	− 3.0	− 2.3	− 4.0	− 5.0
6	No	4.30	3.90	—	—	−3.5	−3.8	—	—	−7.0	− 5.6	− 6.5	− 4.6	− 7.5	− 1.3	—	—
7	No	4.50	4.12	—	—	−2.5	−7.0	—	—	−5.6	− 7.0	− 1.1	− 6.8	—	—	—	—
8	Yes	4.67	3.00	−2.0	+ 3.2	−2.0	+6.6	−1.7	+ 9.3	−1.5	+10.0	− 3.0	+12.6	− 3.7	+13.2	− 6.0	+16.6

* Blood volume in these experiments varied from 110–130 cc.
† R is RBC count ($\times 10^6$) per mm.^{3}.
‡ L is the absolute lymphocyte count ($\times 10^3$) per mm.^{3}.
§ Blood sugar maintained by injection of 18–24 mg. glucose every 30 minutes into the pump.

periments, after a preliminary period of spleen perfusion at a mean arterial pressure of 100 mm. Hg. the arterial pressure was increased to 150–200 mm. Hg. Following the increase in arterial pressure, the number of circulating lymphocytes uniformly rose while the level of circulating erythrocytes was not affected. In three experiments, the arterial pressure was then decreased to 100 mm. Hg. after the spleen had discharged lymphocytes in response to the increase in the arterial pressure. In no case during the subsequent two-hour period did the level of circulating lymphocytes fall when the perfusion pressure was decreased. Fig. 3 shows a typical experiment of this group, illus-

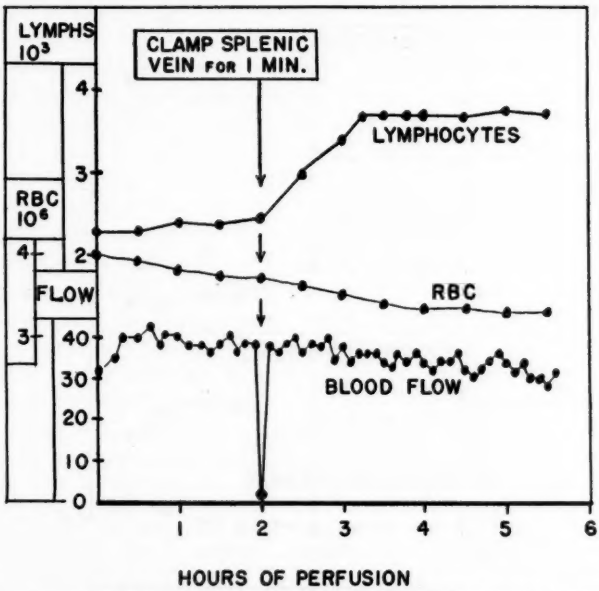


FIG. 4. The effect of temporary obstruction of the splenic vein of a perfused spleen upon the number of circulating lymphocytes and erythrocytes. All values in terms of cells per mm.^{3}. Blood flow in cc. per min.

trating the effect of first increasing, then decreasing the perfusion pressure of an isolated spleen upon the level of circulating lymphocytes.

To determine whether temporary increase of venous pressure would likewise lead to lymphocyte discharge from spleen, in two experiments the splenic vein of an isolated spleen was clamped for one minute and then released, while the organ was being perfused at a constant pressure. As a result of the venous obstruction, the splenic volume markedly increased during the period of venous obstruction, and then returned to normal following removal of the venous clamp. In both experiments the level of circulating lymphocytes, following

temporary venous obstruction, increased to a maximal value which was then maintained, and here again, the level of circulating erythrocytes was not affected. Fig. 4 shows one of these experiments illustrating the effect of temporary venous obstruction of a perfused spleen upon the level of circulating lymphocytes.

These results demonstrate that under the influence of increased arterial or venous pressure, the rate of lymphocyte discharge from

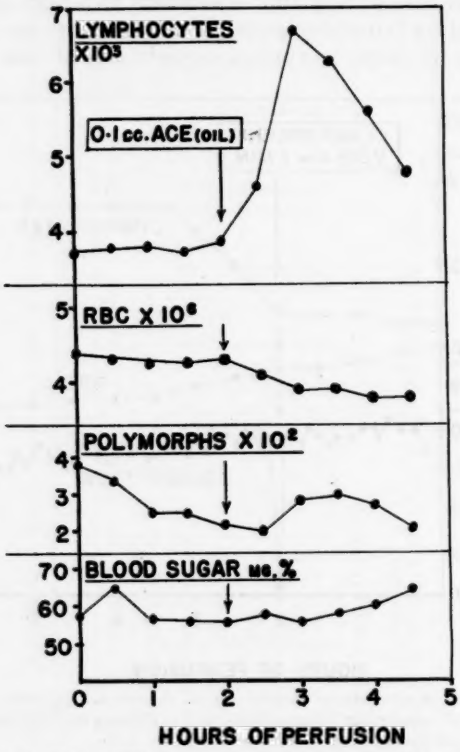


FIG. 5. The effect of adding ACE to a perfused spleen, upon the cellular components of blood. All values in terms of cells per mm.³.

spleen is accelerated so that the level of circulating lyphocytes rises. During a 2-hour interval following the removal of the initiating stimulus, the level of circulating lymphocytes as the result of increased pressure was maintained and showed slight or no tendency to fall. It may therefore be inferred that the lymphocytes discharged by spleen because of increased pressure do not return to the spleen to be retained, nor does it appear that the initial rate of destruction of circulating lymphocytes secondarily increases after splenic lymphocytes are discharged.

Effect of Addition of ACE upon the Perfusion Spleen. In the next experiments the effect of adding ACE (oil or aq.) to the spleen perfused with a constant arterial pressure was studied. Fig. 5 shows the results of a representative experiment illustrating the effect of ACE (oil) upon the cellular elements in the perfusion medium. In this experiment the blood pressure varied between 90 and 100 mm. Hg., the blood sugar was maintained at approximately 60 mg. per cent by administration of glucose (15 mg. at 30 minute intervals); all extrasplenic blood vessels were ligated and after two hours of perfusion, 0.1 cc. of ACE (oil) was added to the system. It will be seen that following the addition of ACE, the number of circulating lymphocytes rapidly increased and then fell while the level of circulating erythrocytes or polymorphonuclear leucocytes was not affected.

Table 3 shows the composite results of 11 experiments on spleen perfused with constant mean pressure of 100 ± 10 mm. Hg. in which the effect of ACE upon the level of circulating lymphocytes was studied, in contrast to other experiments, to be mentioned later, where other agents in addition to ACE were tested. Since the levels of circulating erythrocytes or polymorphonuclear leucocytes were not influenced as the result of addition of ACE to the system, only the changes in circulating lymphocytes are shown. It will be seen that the addition of ACE to the perfused isolated spleen, was consistently followed by an increase in the number of circulating lymphocytes, amounting on the average to 1.60×10^3 lymphocytes. The splenic discharge of lymphocytes induced by ACE is uniformly observed despite the fact that the conditions employed in the individual experiments differed in several respects. From Table 3 it is apparent that the *in vitro* effect of ACE does not appear to be influenced *qualitatively* by the following factors:

(a) *Blood sugar level:* Compare experiments 2, 3, 5, 7, 8 to 1, 4, 6, 9, 10, 11.
(b) *Circulatory differences in the organ as the result of leaving certain non-splenic blood vessels open or closed:* Compare experiments 1–6 to 7–11.
(c) *Initial level of circulating lymphocytes:* Compare experiment 1 to experiment 6 or 8.
(d) *Nature of the vehicle:* Compare experiments 1, 2, 3, 4, 5, 8 to experiments 6, 7, 9, 10, 11. The conclusion that the vehicle for the ACE is not an important factor was substantiated further by observations that cottonseed oil or that small concentrations of chlorobutanol (the components of the oil vehicle for the lipo-adrenal extract) when tested alone or together had no influence on the isolated spleen.
(e) *Presence or absence of antibiotics:* Compare experiments 9 and 11 to the other experiments.

It should be emphasized that it is possible that any or all of the above factors may affect the *quantitative* aspects of the reaction wherein ACE produces lymphocyte discharge from the spleen. This possibility, however, remains for future investigation. At the present

TABLE 3. THE EFFECT OF ADRENAL CORTEX EXTRACT UPON THE ABSOLUTE LYMPHOCYTE COUNT OF THE PERFUSION BLOOD CIRCULATED THROUGH AN ISOLATED SPLEEN

Exp. no.	Wet wt. spleen	Blood vol.	Blood sugar maintained*	Splenic circulation†	Antibiotics added	Lymphocyte count ($\times 10^3$) per mm.^{3} after addition of spleen to pump							ACE administered‡		Lymphocyte count ($\times 10^3$) per mm.^{3} after addition of ACE							Absolute lymphocyte discharge§ $\times 10^3$
						0 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	Vehicle	Dose	0 min.	15 min.	30 min.	45 min.	60 min.	90 min.	120 min.	
	g.	cc.												cc.								
1	2.53	130	No	Open	Yes	4.12		3.83		3.66	3.82	3.80	Oil	0.6	3.80	4.75	5.95	6.44	7.00	6.45	6.15	4.16
2	2.12	132	Yes	Open	Yes	2.20	2.18	2.16		2.20	2.15	2.14	Oil	0.5	2.14	2.63	3.00	2.59	2.28	2.14		1.14
3	1.91	144	Yes	Open	Yes	2.28	2.50	2.83		2.78	2.84	2.90	Oil	0.5	2.90	2.98	3.56	3.30	3.40			0.95
4	3.85	138	No	Open	Yes	2.67	2.78	2.90	3.02	3.08			Oil	0.1	3.08		4.05	4.45	3.76	3.21	2.94	1.86
5	3.54	130	Yes	Open	Yes	3.68	3.80	3.80	3.74	3.93			Oil	0.1	3.93		4.53	6.68	6.40	6.35	6.10	3.58
6	2.54	110	No	Open	Yes	1.51	1.25	1.13	1.17	1.22			Aq.	1.0	1.22		1.48	1.79	2.16	2.10		1.03
7	2.86	145	Yes	Closed	Yes	2.57	2.42	2.61	2.70	2.74			Aq.	1.0	2.74	3.23	3.48	3.01	2.79	2.70		1.07
8		117	Yes	Closed	Yes	1.52	1.51	1.54		1.39			Oil	0.1	1.39	1.47	1.68	2.56	2.00	1.93		1.40
9	1.99	150	No	Closed	No	2.25	2.30	2.27	2.20	2.30			Aq.	1.0	2.30	2.45	2.70	3.10	3.00	2.50		1.20
10	2.02	120	No	Closed	Yes	2.05	2.09	2.00	2.09	2.13			Aq.	1.0	2.09	2.63	2.58	2.60	2.48	2.15		0.65
11	2.54	140	No	Closed	No	2.71	2.70	2.73					Aq.	2.0	2.73		3.00		3.17	3.10	2.70	0.62

* Blood sugar maintained (70–100 mg. %) by injection of 18–24 mg. glucose every 30 minutes.
† Refers to whether splenic preparation had bleeders (open) or whether all non-splenic arterial vessels were ligated (closed).
‡ Hormone administered into pump, after the last sample in the control period was taken.
§ Splenic lymphocyte discharge induced by ACE estimated from the highest post-injection value, as compared to the initial lymphocyte level.

time, mention is only made of the fact that from a *qualitative* point of view, these diverse factors do not appear to influence the *in vitro* effect of ACE upon spleen to produce lymphocyte discharge.

It will be seen from Table 3 that following the initial rise in circulating lymphocytes induced by addition of ACE to spleen, there is a tendency for the level of circulating lymphocytes to fall. Thus, in the eleven experiments shown in Table 3, the circulating lymphocytes return to the pre-ACE treatment value in four cases, in six experiments the return to the initial level is partial, while the increased level

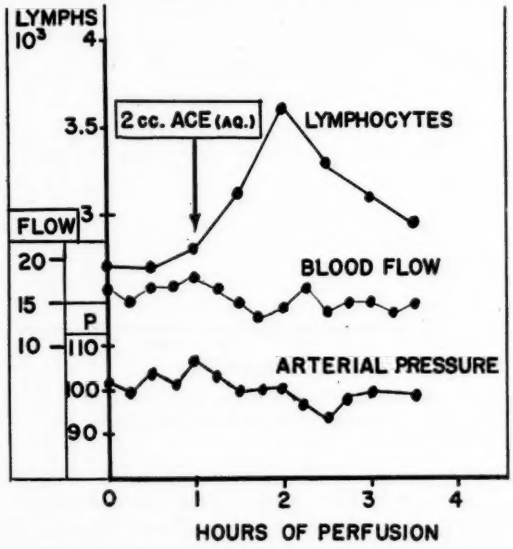


FIG. 6. Blood flow, arterial pressure, and circulating lymphocytes in a perfused spleen before and after addition of ACE. Lymphocytes per mm.³. Blood flow in cc. per min., P-Arterial pressure in mm. Hg.

of circulating lymphocytes was maintained without a fall in only one of the eleven experiments.

To determine whether the secondary fall in circulating lymphocytes observed with ACE could be accounted for as the result of retention of circulating lymphocytes by the spleen, the splenic preparations perfused with ACE were sectioned and examined histologically. In this study the spleens were divided into three groups on the following basis: (a) a complete return of the lymphocyte level to the initial value, (b) only a partial return, and (c) no subsequent fall in the level of circulating lymphocytes. Histological examination revealed no significant difference between these three groups of spleens as regards the total number of lymphocytes in spleen. Thus, it would appear that the secondary fall of circulating lymphocytes observed

with ACE is not explicable on the basis that the decrease is due to return and retention of circulating lymphocytes in the spleen. In all groups, the histological picture was qualitatively similar to that previously described for spleens perfused in the absence of added ACE, although there did appear to be a definite tendency for the ACE treated spleens to show greater lymphocyte depletion.

Effect of ACE upon Blood Flow through Spleen. To determine whether the effect of ACE upon the splenic discharge of lymphocytes was associated with a change in blood flow through the spleen, measurements of spleen blood flow were made in three experiments, before,

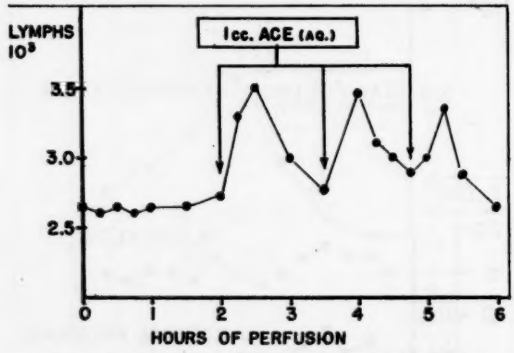


FIG. 7. The effect of repetitive addition of ACE upon the level of circulating lymphocytes (per mm.³) in an isolated perfused spleen.

during, and after ACE induced splenic discharge of lymphocytes. In no instance did ACE produce a significant alteration of spleen blood flow. Fig. 6 illustrates a typical experiment which demonstrates that ACE induces the typical rise and fall of circulating lymphocytes without significant alteration of splenic blood flow.

Repetitive Effect of ACE upon Spleen. The effect of repetitive addition of ACE was determined in two experiments. In both cases, it was observed that after the first dose of ACE had produced a rise and fall of circulating lymphocytes, injection of another dose of ACE produced a similar response. Fig. 7 illustrates an experiment wherein three doses of ACE given separately produced three similar responses of lymphocyte discharge from spleen, followed by subsequent removal or destruction, of circulating lymphocytes.

Effects of Other Agents upon the Isolated Spleen. Fig. 8 shows a representative experiment of a group of three illustrating the ineffectiveness of 1 mg. amounts of either estradiol dipropionate or desoxycorticosterone acetate to produce the splenic discharge response to an isolated spleen preparation which has the capacity to respond to ACE (oil) Fig. 9 shows a representative experiment of a group of three illustrating the lack of effect of 100 mg. glucose or 0.1 mg. epinephrine

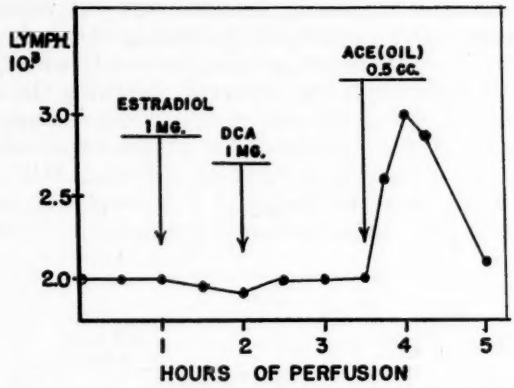


FIG. 8. The effect of adding estradiol dipropionate, desoxycorticosterone acetate, and ACE upon the level of circulating lymphocytes (per mm.³) in a perfused spleen system.

hydrochloride (1 cc. of 1:10,000) administered into the venous side of the pump upon the splenic discharge reaction. It will be seen that while neither agent influenced the level of circulating lymphocytes, addition of ACE (aq.) produced the usual discharge reaction in the same spleen preparation. In two additional experiments, 0.001 mg. epinephrine hydrochloride failed to induce splenic lymphocyte discharge.

The Effect of ACE upon other Isolated Organs. To determine whether the *in vitro* effect of ACE upon spleen was specific for that organ, the effect of ACE upon thymus lung, and liver were next studied.

In eight experiments where 0.5 cc. of ACE (oil) was added to perfused thymus preparations, it was uniformly observed that ACE never produced an increase in the number of circulating lymphocytes. In six of the experiments with thymus, no effect of ACE addition

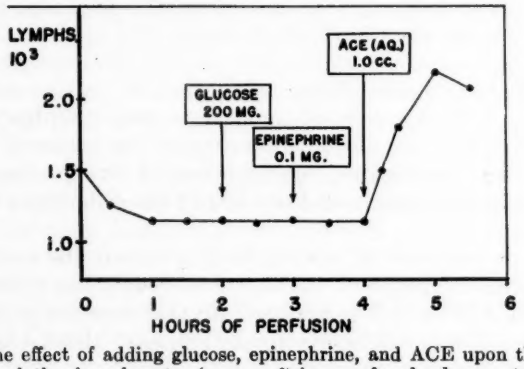


FIG. 9. The effect of adding glucose, epinephrine, and ACE upon the level of circulating lymphocytes (per mm.³) in a perfused spleen system.

upon the number of circulating lymphocytes was noted. Fig. 10a shows a representative experiment illustrating this lack of effect of ACE upon the level of circulating lymphocytes. However, in two experiments with thymus, it was observed, following the addition of ACE, that the circulating lymphocytes practically disappeared from the perfusion blood. Fig. 10b shows one of these experiments. No explanation for the differences in response following ACE addition to thymus is at hand, since the dosages of ACE employed, and all other known variables were similar in these experiments. It was noted, how-

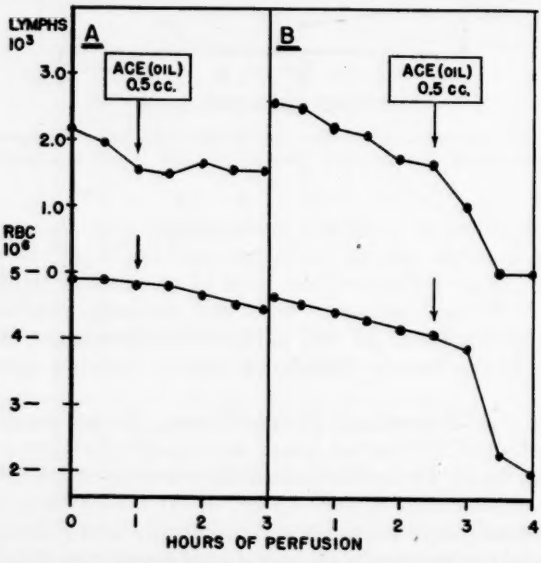


FIG. 10. The effect of adding ACE upon the cellular components of blood perfused through thymus gland. *10 A* represents the usual picture wherein ACE produces no significant effect. *10 B* represents one of two experiments wherein ACE produced a marked fall in circulating lymphocytes associated with a decrease in the number of circulating RBC. All values in terms of cells per mm.³.

ever that in both cases where ACE addition was associated with marked reduction of the number of circulating lymphocytes, a significant decrease of circulating erythrocytes also occurred. It may be mentioned that histological examination of the perfused thymus glands in these experiments did not clarify the differences in response noted.

While it is not possible to completely evaluate the action of ACE addition upon the perfused thymus, one conclusion seems definite: namely, that ACE added to perfused thymus does not produce a discharge of lymphocytes into the circulation, as evidenced by measurements of circulating lymphocytes.

Using lung in four experiments and liver in four experiments, it was consistently observed that the addition of 0.5 to 1.0 cc. ACE (oil) did not lead to any significant effect upon the number of circulating lymphocytes. Fig. 11 shows a representative experiment of lung and also a representative experiment on liver.

These negative results on the effect of ACE upon thymus, lung,

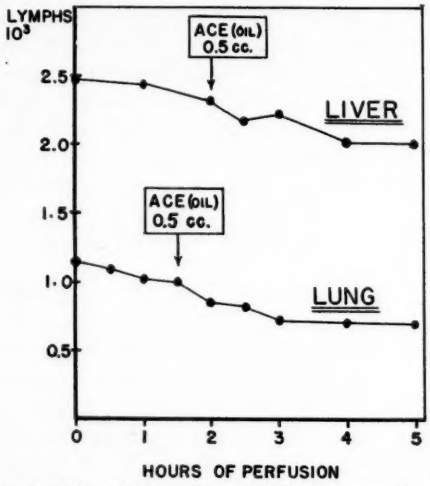


FIG. 11. The effect of adding ACE to lung and liver, upon the level of circulating lymphocytes (per mm.^{3}) in the perfusion medium.

and liver suggest that the effect of ACE to produce lymphocyte discharge from tissues is specific for spleen.

DISCUSSION

These studies demonstrate that the addition of ACE to isolated rabbit spleen, perfused with heparinized blood at constant pressure, induces first a rise in the level of circulating lymphocytes, which secondarily tends to fall to the pre-treatment level. The rise in the number of circulating lymphocytes induced by ACE can be explained only on the basis of a splenic discharge of lymphocytes. This ACE induced splenic discharge phenomenon is rapid, occurring within 15 minutes, and reaches its maximum in 30 to 60 minutes.

In contrast to the rise in circulating lymphocytes, the reason for the secondary fall of lymphocyte level in the ACE treated spleen which occurs within a period of 2 hours of ACE administration, is less clear cut.

The secondary fall in lymphocyte level observed after ACE induced splenic discharge appears to be due to ACE, since splenic lymphocyte discharge produced as the results of increased arterial or venous pressure is not associated with a fall in the circulating lympho-

cyte level when the pressure stimulus is removed. It, therefore, appears that ACE induces the secondary fall in circulating lymphocytes either by facilitating (a) the return and retention or (b) the breakdown of lymphocytes in the isolated spleen. Histological examination of spleens perfused with ACE demonstrated consistent splenic lymphocyte depletion, independent of whether the secondary fall was absent, partial or complete. This histological finding thus strongly suggests that the secondary fall of lymphocytes observed with ACE is due to an increased breakdown of circulating lymphocytes by the perfused spleen. This evidence would seem to indicate that the well established *in vivo* action of ACE to increase the rate of lymphocyte dissolution in lymphoid structures (White and Dougherty, 1946) occurs under *in vitro* conditions of splenic perfusion. However, it should be emphasized that before it can be definitely stated that ACE accelerated the breakdown of lymphocytes under *in vitro* conditions of splenic perfusion, direct evidence of this process would be necessary.

The effect of ACE to induce *in vitro* splenic lymphocyte discharge could not be duplicated by estradiol dipropionate, desoxycorticosterone acetate, glucose or epinephrine, in the dosages tested. It is fully recognized that further work using a variety of steroids, as well as other agents, tested at varying dosage levels, will be necessary before the absolute specificity of the splenic discharge reaction to ACE will be delineated. However, these preliminary *in vitro* studies demonstrate that not all steroids produce the reaction, and that desoxycorticosterone acetate, which does not affect lymphoid structures or blood lymphocyte levels *in vivo* (White and Dougherty, 1946) likewise did not produce the splenic lymphocyte response.

Concerning the failure of epinephrine to induce discharge of splenic lymphocytes, several features of the experimental conditions employed *in vitro* must be considered before final conclusions are drawn. It should be noted that only two dosages of epinephrine were tested in a limited number of experiments; and that the epinephrine was administered into the venous side of the pump. The possibility therefore arises that the administered epinephrine was completely inactived in the perfusion blood before it could enter the spleen to act. Furthermore, it is known that certain end organ responses to epinephrine are qualitatively dependent upon the dosage of epinephrine (Grollman, 1936). It should be stressed that epinephrine in these experiments was tested solely to determine whether the effect of ACE upon the perfused spleen could be explained in terms of a possible epinephrine contamination of the extract. Our negative results with epinephrine have significance to the extent that they suggest that ACE action upon splenic discharge of lymphocytes cannot be explained on this basis. A detailed study of the effect of epinephrine upon *in vitro* splenic lymphocyte discharge using varied dosages, administered into the arterial circulation, remains for future investi-

gation, and may well reveal that epinephrine, as well as ACE, promotes this splenic reaction. Moreover, the fact that splenic lymphocyte discharge can be produced *in vitro* via increased perfusion pressure, suggests that epinephrine might produce splenic discharge of lymphocytes *in situ* via its pressor activity.

Regarding the specificity of ACE to induce lymphocyte discharge from other perfused tissues, it has been demonstrated that addition of ACE to perfused thymus, liver, or lung did not induce lymphocyte discharge. The finding that ACE did not discharge lymphocytes from a lymphoid structure such as thymus suggests that the ability of ACE to effect lymphocyte discharge may be related to the fact that splenic lymphocytes normally enter the blood directly, without first passing into the lymph stream, in contrast to the lymphocytes from other lymphoid structures.

What can be stated regarding the mechanism of ACE action to produce lymphocyte discharge from the isolated spleen? The results of this study throw light on this problem only in a negative way. Thus, the *in vitro* effect of ACE to produce splenic discharge can be produced without alteration of splenic blood flow or mean arterial pressure; nor has an obvious splenic contraction been visualized in association with this reaction. Whether the ACE effect to produce lymphocyte discharge from spleen is due to alteration in intrasplenic circulation, to splenic contraction, or to the release of lymphocytes from tissue elements to which they are fixed are questions which remain to be elucidated.

One aspect of our results upon the isolated rabbit spleen, not directly related to ACE action, merits discussion. It will have been noted that in all circumstances where splenic lymphocytes were discharged, whether by ACE at constant arterial pressure or as the result of increased splenic arterial or venous pressures, in no instance was there noted a coincident discharge of erythryocytes sufficient to increase the circulating red blood cell count. This finding, at first sight, appears to be in contradiction to the well established blood reservoir function of the spleen, whereby splenic contraction forces blood from this depot into the active circulation (Barcroft, 1926, 1930).

It should be recalled that the increase in circulating RBC observed *in vivo* (in some species) associated with splenic contraction is possible only because the blood stored in the spleen is richer in erythrocytes than the circulating blood. In our *in vitro* experiments with the isolated spleen, this situation probably does not exist for the following reasons: the splenic blood, originally present was first removed by the splenic contraction which occurred upon the death of the animal. Thus, the spleen inserted into the perfusion pump is in its contracted state with no concentrated blood. Upon initiation of circulation through the isolated spleen, the possibility of reconcentrating erythrocytes from the perfusion blood probably is limited due to the absence of regulated nervous control of splenic blood flow. *In situ*, blood

concentration in the spleen seems to be dependent upon rhythmic splenic contractions induced by regulated blood flow which causes the fluid elements of blood to be forced into lymphatic vessels while retaining the formed elements of blood in the red pulp (Hamilton, 1946). It, therefore, seems likely that the failure to observe erythrocyte discharge in our experiment is not due to the absence of this process but to the fact that it is not measurable because of lack of RBC concentration in the perfused spleen.

SUMMARY

The administration of adrenal cortex extract to the isolated rabbit spleen, perfused with whole blood at a constant mean pressure, induces a prompt discharge of splenic lymphocytes into the circulation. There is, secondarily, a tendency for the level of circulating lymphocytes in the perfusion medium to fall. The secondary decrease in lymphocyte count appears to be due to accelerated lymphocyte breakdown by spleen, in the presence of ACE. The activity of ACE to produce the *in vitro* reaction upon spleen is not associated with alteration of mean arterial pressure or changes in splenic flow, and can be demonstrated upon repetitive addition of ACE. The addition of ACE to perfused thymus, liver, or lung does not produce discharge of lymphocytes from these tissues; glucose, epinephrine, desoxycorticosterone acetate, and estradiol dipropionate in the doses tested, did not provoke lymphocyte discharge from spleen.

ACKNOWLEDGMENTS

Thanks are due Dr. G. Pincus for valuable suggestions and encouragement; Mrs. E. Scully and Miss M. Aubertine for technical assistance; Dr. N. Werthessen for suggestions regarding certain features of the perfusion apparatus and to Drs. E. Dempsey and G. D. Kaneb for examining the histological sections. In addition, we wish to thank Dr. M. Kuizenga, Upjohn Co., Kalamazoo Michigan, for generously supplying us with heparin sodium and the lipo-adrenal extract, and Dr. Weiner, Endo Products, N.Y., for the aqueous adrenal cortical extract.

REFERENCES

BARCROFT, J.: *Lancet* 1: 544. 1926.
BARCROFT, J.: *J. Physiol.* 68: 375. 1930.
CARREL, A., AND C. A. LINDBERG: Culture of Organs, P. B. Hoeber, New York, 1938.
GROLLMAN, A.: The Adrenals, Williams & Wilkins, Baltimore, p. 113, 1936.
HAMILTON, W. F.: Howell's Textbook of Physiology, edited by J. F. Fulton, W. B. Saunders, Philadelphia, p. 846, 15th edit. 1946.
HEMINGWAY, A.: *J. Physiol.* 71: 201. 1931.
JAQUES, L. B., E. FIDLAR, E. T. DELDSTED AND A. G. MACDONALD: *Canad. M. A. J.* 55: 26. 1946.
LINDBERGH, C. A.: *J. Exper. Med.* 62: 409. 1935.
ROCHE E SILVA M., A. E. SCROGGIE, E. FIDLAR AND L. B. JAQUES: *Proc. Soc. Exper. Biol. & Med.* 64: 141. 1947.
SCHALES, O., AND S. S. SCHALES: *Arch. Biochem.* 8: 285. 1945.
WHITE, A., AND T. F. DOUGHERTY: *Ann. N. Y. Acad. Sci.* 46: 850. 1946.

SPLENIC LYMPHOCYTE DISCHARGE INDUCED BY ADRENAL CORTICAL HORMONES UNDER IN VIVO CONDITIONS[1]

DAVID STONE AND OSCAR HECHTER

From the Worcester Foundation for Experimental Biology, and the Department of Physiology, Tufts Medical School

BOSTON, MASS.

IN A PRECEDING PAPER, it was demonstrated that adrenal cortex extract (ACE) produces lymphocyte discharge from the perfused rabbit spleen (Hechter, 1948). It was therefore of interest to determine whether ACE produces a similar reaction upon the spleen under *in vivo* conditions. In this connection, our attention was drawn to the fact that some stresses appear to be associated with an absolute lymphocytosis, instead of the lymphopenia which has been more commonly associated with increased adrenal cortical activity (cf. White and Dougherty, 1946). Thus, absolute lymphocytosis has been reported following: exercise in rats and mice (Harlow and Selye, 1937) and in man (cf. Garrey and Bryan, 1935), short-term (five minute) restraint in rats (Farris, 1938b), and so-called "emotional stimulation" in man (Farris, 1938a).

In view of our *in vitro* findings, it seemed possible that the lymphocytosis associated with these stress situations might in part result from a possible action of adrenal cortical hormone (ACH) upon the splenic lymphocyte discharge mechanism. To test this hypothesis, a preliminary investigation was undertaken to find a stress situation which would produce a significant degree of lymphocytosis. Since both exercise and so-called "emotional stimulation" in rats produce only slight rises in the absolute number of circulating lymphocytes, the effect of combining both stimuli by forcing animals to swim was studied. It was then observed that swim-stress in rats consistently produced a significant lymphocytosis. Accordingly, the effect of splenectomy and adrenalectomy upon this response was determined. In addition, the effect of ACE and desoxycorticosterone acetate (DCA) in adrenalectomized and adrenalectomized-splenectomized rats subjected to swim-stress was studied.

METHODS

Male albino rats, of the Sherman strain raised in this laboratory, weighing from 180 to 200 grams and fed a diet of Purina dog chow, were used in

Received for publication February 24, 1948.

[1] Aided in part by a grant from the G. D. Searle and Company and by Contract N6ori-197 of the Office of Naval Research.

these studies. The adrenalectomized animals were prepared in the usual fashion, and after operation were given the stock diet plus 1 per cent NaCl in the drinking water. The animals were used for experimentation 2–3 days after adrenalectomy. Splenectomy was performed through a dorsal incision, 24 hours before experimentation, after which time these animals received no special treatment. For swim-stress, rats were placed in a bath-tub containing water at 27°–28C. They were removed from the tub for blood sampling or injections. Blood samples were obtained from the tail; white and red blood cells were measured in the usual fashion; and differential leucocyte counts were made by counting 100 cells on the air-dried smears, stained with Wright's stain.

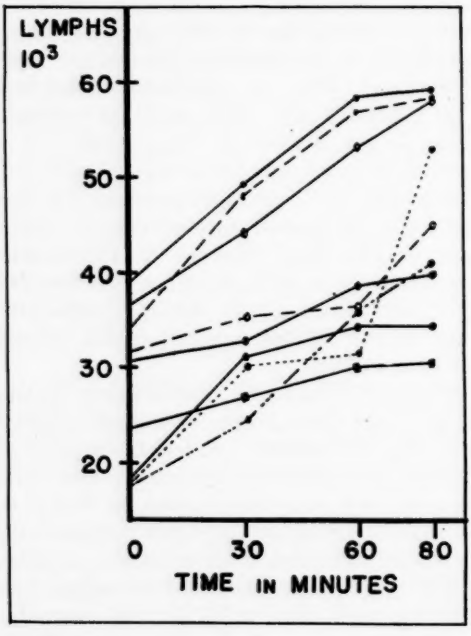


FIG. 1. The effect of forced swimming of normal rats upon the absolute lymphocyte level (per mm.^{3}) of the blood. Each curve represents a single animal and it will be noted that absolute lymphocytosis regularly develops.

RESULTS

Fig. 1 shows the effect of swimming normal rats upon the absolute level of circulating lymphocytes. It will be seen that an absolute lymphocytosis uniformly develops. Table 1 illustrates the average results in four rats subjected to swim-stress, in which circulating lymphocytes and erythrocytes were simultaneously measured. It will be observed that the absolute lymphocytosis of swim-stress in normal rats is not due to hemoconcentration.

Table 2 illustrates the comparative results obtained upon sub-

jecting adrenalectomized, splenectomized, and normal rats to swim-stress. The results indicate that in normal rats there is a significant absolute lymphocytosis after 60 to 80 minutes of forced swimming. Following adrenalectomy, the lymphocytosis of swim-stress is abolished. Splenectomy significantly reduces the lymphocytosis response at 60 and 80 minutes (P values <0.01 as compared to normals). While the splenectomized rats subjected to swim-stress do exhibit some increase in circulating lymphocytes over their initial blood levels, this rise, it should be pointed out, is not statistically significant. Simul-

TABLE 1. EFFECT OF SWIMMING NORMAL RATS UPON THE PERCENTAGE CHANGE OF LYMPHOCYTES AS COMPARED TO ERYTHROCYTES

Rat no.		Percentage change	
		30 min.	60 min.
1	RBC	+21	+14
	L*	+38	+28
2	RBC	+15	+ 3
	L	+71	+85
3	RBC	+ 8	−15
	L	+58	+86
4	RBC	+24	+30
	L	+89	+80
Mean	RBC	+19	+ 8
	L	+64	+70

* Lymphocytes.

taneous counts of circulating lymphocytes and RBC in adrenalectomized or splenectomized rats forced to swim for 80 minutes revealed no significant changes from the initial values.

The next experiments were designed to test whether ACE or DCA could induce lymphocytosis in swimming adrenalectomized animals. In these experiments the rats were first forced to swim for 30 to 40 minutes, and then either 0.25 cc. of lipoadrenal cortex extract (Upjohn) or DCA (5.0 mg. per cc.) was injected intraperitoneally. After injection, the rats were returned to the water bath and forced to swim for another 60 minutes. Blood samples were obtained immediately before, and 30 to 60 minutes after the injection of ACE or DCA. Table 3 shows the results obtained. It will be observed that 60 minutes after ACE treatment swimming adrenalectomized rats develop a blood lymphocytosis. In contrast, DCA fails to induce lymphocytosis. Simultaneous red blood cell counts demonstrated that injection of ACE or DCA in swimming adrenalectomized rats was not associated with either hemo-dilution or hemo-concentration.

TABLE 2. THE EFFECT OF SWIM-STRESS UPON THE ABSOLUTE LYMPHOCYTE LEVEL IN NORMAL, SPLENECTOMIZED AND ADRENALECTOMIZED RATS

No. of rats	Type	Period of forced swim											
		0 min.	30 min.				60 min.				80 min.			
		L*	L	Diff.	P_1†	P_2‡	L	Diff.	P_1	P_2	L	Diff.	P_1	P_2
10	Normal	27.3 ±2.6§	35.7 ±3.0	% +31	0.05	—	41.8 ±3.8	% +53	<0.01	—	44.2 ±4.0	% +62	<0.01	—
11	Splenectomized	29.2 ±3.5	35.8 ±4.7	+23	0.2 to 0.3	0.1	36.6 ±4.3	+25	0.2	<0.01	36.1 ±4.9	+24	0.2 to 0.3	<0.01
12	Adrenalectomized	22.1 ±2.3	23.6 ±2.3	+ 7	>0.5	<0.01	22.0 ±2.1	+0.2	>0.5	<0.01	21.2 ±2.8	+ 4	>0.5	<0.01

* Is the absolute lymphocyte count ($\times 10^3$) per mm.^{3}.
† P_1 is the "p value," comparing mean lymphocyte levels after swim to pre-swim in the same group of rats.
‡ P_2 is the "p value," where the mean lymphocyte changes in swimming splenectomized or adrenalectomized rats is compared to the lymphocyte change in normal rats swum for the same periods of time.
§ Standard error of the mean.

To determine whether the lymphocytosis produced by ACE in swimming adrenalectomized rats was due to discharge of splenic lymphocytes into the circulation or to a possible mobilization of extrasplenic lymphocytes, the effect of ACE was tested in swimming adrenalectomized-splenectomized rats. In these experiments, 0.25 cc. ACE was injected intraperitoneally into the rats after they had been forced to swim for 30 to 40 minutes. These results are likewise shown in Table 5 from which it will be seen that ACE administered to swimming adrenalectomized-splenectomized rats does not produce lymphocytosis; indeed there is a tendency for ACE to produce lymphopenia under these conditions.

In an attempt to obtain direct evidence that splenic lymphocytes are discharged into the circulation under conditions of swim-stress,

TABLE 3. THE EFFECT OF ADRENOCORTICAL HORMONES UPON THE ABSOLUTE LYMPHOCYTE COUNT IN ADRENALECTOMIZED AND SPLENECTOMIZED-ADRENALECTOMIZED RATS, SUBJECTED TO SWIM STRESS

No. of rats	Type	Treatment	Time after injection						
			0 min.	30 min.			60 min.		
			L*	L	Diff.	P†	L	Diff.	P†
21	Adrenalectomized	ACE‡	24.7±1.69§	27.9±1.63	+13%	>0.05	31.2±1.69	+26%	<0.01
8	Adrenalectomized-Splenectomized	ACE‡	23.4±1.43	22.2±1.26	− 5	>0.5	2.06±1.03	−12	0.1–0.2
7	Adrenalectomized	DCA‖	25.0±2.73	28.2±4.0	+13	>0.5	21.8±3.26	−13	0.5

* L is the mean absolute lymphocyte count ($\times 10^3$) per mm.^{3}.
† P is the "p value" where the mean change in lymphocytes after injection is compared to pre-injection value.
‡ 0.25 cc. lipo-adrenal extract injected intraperitoneally.
§ Standard error of the mean.
‖ 0.25 cc. of desoxycorticosterone acetate (5 mg./cc.) administered intraperitoneally.

the number of lymphocytes extractable from spleen were measured in normal, unstressed rats and matched rats which were forced to swim for 60 minutes. To check the hypothesis that splenic discharge fails to occur in the absence of the adrenals, similar experiments were made comparing the number of splenic lymphocytes in non-stressed adrenalectomized rats with those of adrenalectomized rats forced to swim for 60 minutes. In these experiments, the spleen was removed from etherized rats, weighed, ground to a mash with saline in a mortar, and then transferred to a volumetric flask. To destroy the RBC present in the extract, 10 cc. of 0.1N HCl was added, the volume was adjusted to 100 cc., and WBC counts were made in the usual manner. It was assumed that all of the WBC were lymphocytes since smears made on the mortar mash revealed that practically all of the leucocytes in the spleen extracts were lymphocytes. Fig. 2 shows the effect of swimming normal and adrenalectomized rats upon the total number of lymphocytes extractable from the spleen. It will be seen that normal animals, subjected to swim-stress, exhibit an average decrease of 27 per cent in the number of splenic lymphocytes from the initial unstressed value, while swim-stressed adrenalectomized rats show only

an average decrease of 7 per cent. While these findings tend to agree with the view that splenic discharge occurs in normal animals, but not in adrenalectomized rats, subjected to swim-stress it should be emphasized that the results are not statistically significant. Whether the variability in the results observed is due to the method employed or is inherent in the nature of the experiment, remains to be eluci-

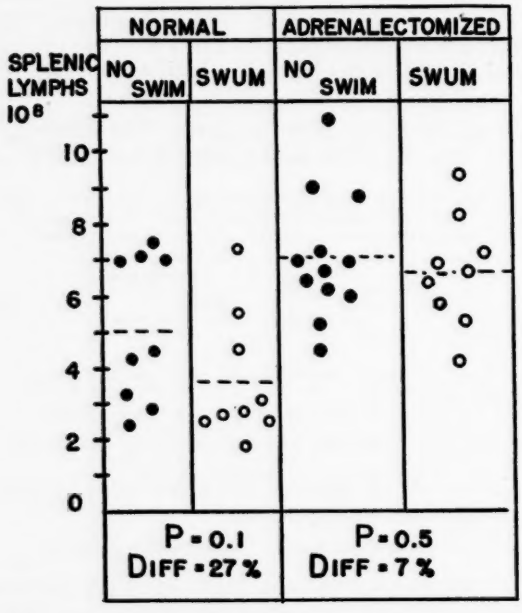


FIG. 2. The absolute number of lymphocytes extractable from spleens of normal and adrenalectomized rats after a 60-minute swim, as compared to non-swum normal and adrenalectomized animals. Each dot or circle represents a single animal. The dotted lines represent the mean values for the various groups.

dated. It may be mentioned, as an incidental finding, that there appears to be a greater number of lymphocytes in the spleens of adrenalectomized rats as compared to normals.

DISCUSSION

These studies strongly suggest that endogenous adrenal cortical hormones induce splenic lymphocyte discharge in rats subjected to swim-stress. Thus, the absolute lymphocytosis associated with forced swimming can be significantly reduced either by splenectomy or adrenalectomy, and can be re-induced in swum-adrenalectomized rats by the administration of ACE only if the spleen is intact. Taken together, with the *in vitro* studies described in the previous paper, these findings provide strong evidence for the view that the control of

splenic lymphocyte discharge by adrenal cortical hormones is a physiological mechanism.

The finding that DCA, in contrast to ACE, cannot induce lymphocytosis in swum-adrenalectomized rats is likewise in agreement with the perfusion studies wherein DCA was ineffective. These results therefore suggest that the splenic lymphocyte reaction which has been discussed is under control of those hormonal corticosteroids which possess an oxygen atom at position 11 of the steroid nucleus, *e.g.* the so-called "sugar-active" cortical hormones. It should be emphasized that the same ACE which induces lymphocytosis in swimming adrenalectomized rats exhibits a potent lymphopenic activity when tested in adrenalectomized rats or mice.

An estimate of the number of lymphocytes discharged from the spleen of swimming rats by administration of ACE may be made by comparison of the lymphocytosis response produced by ACE in swum adrenalectomized and adrenalectomized-splenectomized rats. On the assumption that the rates of peripheral removal of lymphocytes and of extra-splenic lymphocyte delivery of lymphocytes to the blood are the same in both groups, it may be calculated that, on the average, the absolute lymphocyte level is increased 32 per cent by discharge of splenic lymphocytes. Assuming a blood volume of 18 cc. for 200 gram salt-treated adrenalectomized rats (Hechter, 1945) this would represent an average discharge of 1.44×10^8 lymphocytes from rat spleen. This value is approximately 30 per cent of the total number of lymphocytes which we have found to be extractable from the spleens of normal non-stressed rats.

It is of interest to compare the calculated estimates of splenic discharge induced by ACE under *in vitro* conditions from rabbit spleen with the above values. From the perfusion studies described in the preceding paper, it was calculated that approximately 1.60×10^8 lymphocytes are discharged from the average rabbit spleen. Discharge of this number of lymphocytes in a 3 kgm. rabbit (assuming a blood volume of 430 cc. and an initial lymphocyte count of 7000 per cmm.) would increase the blood lymphocyte level only about 5 per cent. In general, it would appear that the importance of ACE-induced splenic lymphocyte discharge upon the blood lymphocyte level among various species would depend to a significant degree upon the ratio of the total number of splenic lymphocytes to the circulating blood volume or, less precisely, to spleen weight/body weight. Species such as the rabbit, where the ratio is low (compared to the rat, for example) should not be expected to exhibit striking alterations in circulating lymphocyte levels as the result of the splenic reaction.

The physiological significance of the splenic discharge reaction, its participation in stresses other than swimming, and its relationship to the well established effect of ACH to produce blood lymphopenia (White and Dougherty, 1946) remains to be evaluated by future work.

It is apparent that the lymphopenic activity of ACH would tend to counteract the effect of the splenic reaction upon the circulating lymphocyte level and that the resultant effect would depend upon the magnitude, latent period and duration of these opposing actions.

SUMMARY

The absolute lymphocytosis produced by swim-stress in normal rats can be significantly decreased either by adrenalectomy or splenectomy. Administration of ACE to swimming adrenalectomized rats produces lymphocytosis; desoxycorticosterone acetate administered under these conditions does not produce lymphocytosis. Administration of ACE to adrenalectomized-splenectomized rats subjected to swim-stress does not produce lymphocytosis. These findings provide strong evidence for the view that ACH induces splenic lymphocyte discharge into the circulation.

ACKNOWLEDGMENTS

We wish to thank Dr. H. Hoagland for valuable suggestions and Mr. R. Grenier for technical assistance.

REFERENCES

FARRIS, J. E.: *Am. J. Anat.* 63: 297. 1938(a).
FARRIS, J. E.: *Am. J. Anat.* 63: 325. 1938(b).
GARREY, W. E., AND W. R. BRYAN: *Physiol. Rev.* 15: 597. 1935.
HARLOW, C. M., AND H. SELYE: *Proc. Soc. Exper. Biol. & Med.* 36: 141. 1937.
HECHTER, O.: *Endocrinology* 36: 77. 1945.
HECHTER, O.: *Endocrinology* 42: 285. 1948.
WHITE, A., AND T. F. DOUGHERTY? *Ann. N. Y. Acad. Sci.* 46: 859. 1946.

THE EFFECT OF REMOVING VARIOUS ENDOCRINE GLANDS ON THE HAIR CYCLES OF BLACK RATS[1]

SALLY H. DIEKE

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, The Johns Hopkins Hospital

BALTIMORE, MARYLAND

THE MANNER in which Norway rats grow their fur and replace it throughout life has been described in a previous paper (Dieke, 1947). It was shown there that in this species the hair follicles near the midventer are the first to become active, then those on the sides and finally those on the head and the rump, so that a complete new coat is acquired in a wave-like fashion, with only the follicles in certain circumscribed areas being active at any given time. Each complete wave of hair growth is called a hair cycle. In black rats the pigment which will color the hair can be seen in the skin just before the hair shafts emerge, which makes it easy to recognize and follow the active phase of each cycle.

In rats of this laboratory the first hair cycle begins several days after birth and is complete at about three weeks of age; the second begins at about one month and is usually completed within another month, while the third cycle begins at about 50 days and takes approximately 2½ months for completion. Subsequent cycles start at increasingly greater intervals and show progressively greater delay in completion; for this reason a half or full grown rat usually has several cycles in progress simultaneously.

A preliminary study has now been made to see whether these hair cycles are under endocrine control. Observations have been made, over periods up to 10 months, on rats surgically deprived of either adrenals, gonads, thyroid (and parathyroids), or hypophysis, with normal (unoperated) litter-mates serving as controls. There were male and female rats in each group.

Except for the adrenalectomized rats, which had access to a bottle of 3 per cent salt solution in addition to their regular food and water, all rats were treated identically, receiving the same standard rations customarily fed in this laboratory and drinking tap water. They were housed in individual cages in a well-ventilated inside room subject to no sharp fluctuations of temperature.

Received for publication February 25, 1948.

[1] Carried out under a contract between the Medical Division, Chemical Corps, U. S. Army, and The Johns Hopkins University. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the author.

The operations were performed when the rats were between 34 and 38 days old. Each rat was then examined carefully twice a week thereafter for fur and skin changes, and the fur was clipped off from one side of the body at weekly intervals, or whenever it had grown sufficiently to obscure the skin pigmentation patterns. The fur on the other side of the body was left undisturbed throughout; it showed no greying or other deviation from normal except that the texture remained soft and juvenile in the hypophysectomized animals.

Completeness of operation was checked in each case at autopsy.

RESULTS

The 2nd hair cycle began at an average age of 30 days (range 28–32 days), before any operations had been performed. Table 1 shows how the various operations acted to delay or advance subsequent cycles.

TABLE 1. AVERAGE AGE OF RATS IN DAYS AT START OF HAIR CYCLES 3 THROUGH 7
(Operations performed at 34 to 38 days of age. Patterns were "normal" after adrenalectomy and gonadectomy, "reversed" after thyroidectomy and hypophysectomy.)

Operation	No. rats	Cycle				
		3rd	4th	5th	6th	7th
None (controls)	2 ♂, 4 ♀	52	81	113	(139–211)	(198–255)
Adrenalectomy	2 ♂, 3 ♀	50	68	93	115	142
Gonadectomy	1 ♂, 3 ♀	57	88	130	205	—
Thyroidectomy	2 ♂, 4 ♀	58	125	200	—	—
Hypophysectomy*	1 ♂, 4 ♀	82	118	179	226	268

* Values given after 3rd cycle are for 1 rat only.

For adrenalectomized rats the 3rd cycle began 2 days sooner than the controls. Subsequent cycles began progressively earlier than normal, so that these rats were starting their 6th cycle at an age (16 weeks) when the control rats had only just begun their 5th. In one case the 12th cycle started before the 7th of a control rat. The rapidity with which the cycles succeeded each other in the adrenalectomized rats, and their coherence compared to later cycles in normal rats, produced the striped effect illustrated in Fig. 1B, where 5 distinct waves of skin pigment appear corresponding to the active growth regions of cycles 7 (in the lumbar region) through 11 (on the belly). Fig. 1A shows for comparison a litter-mate control rat of the same sex, photographed at the same time; here a remnant of cycle 7 appears on the mid-dorsum and fragments of 8 lower on the side.

The gonadectomized rats had hair cycles essentially similar in appearance to those of the controls, and appearing with only slight delay, as may be seen in Table 1. The only marked deviation from a normal appearance of the skin in these rats was in the distribution of the superficial spots of oxidized lipoid material normally found on the backs of male but not of female rats in this colony (Dieke, 1947).

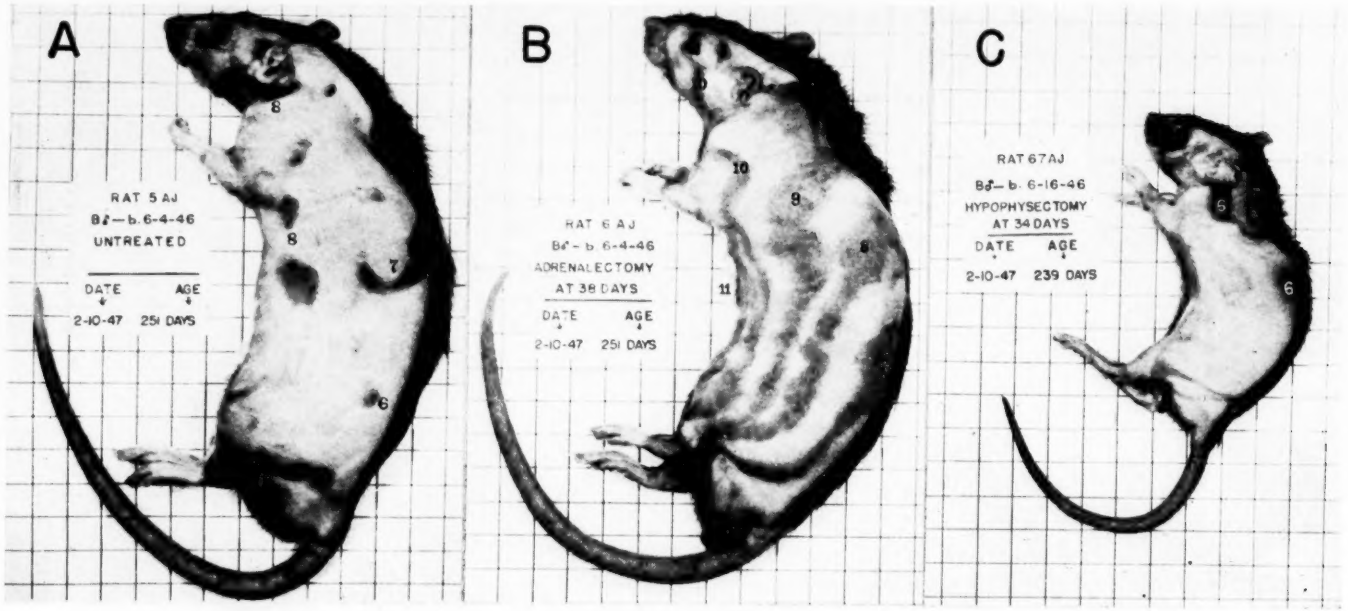


FIG. 1. Appearance of skin in 3 male rats. Fur clipped off in each case just before photographing. Numbers have been added to identify the hair cycles. A. Normal rat, 251 days old; B. Adrenalectomized litter mate to normal rat, also 251 days old; C. Hypophysectomized rat, 239 days old.

The ovariectomized rats showed considerable numbers of these spots, while in the one castrated male they were far fewer than normal, although not entirely absent.

The thyroidectomized rats not only showed great delay in hair replacement, but also had markedly abnormal patterns in those cases judged to have undergone successful operations. The first evidence of a new cycle (the 3rd) after operation did not appear on the belly but rather in a spot on the back of the neck, which then expanded in all directions, so that active growth progressed in a direction exactly contrary to the normal. Two of these rats died at 136 and 189 days of age, without ever starting anything recognizable as a 4th hair cycle. Two others ran through the 4th and 5th cycles, always starting them in the same spot on the back, while the remaining two had greatly delayed cycles but of the normal type. A small remnant of thyroid tissue was found in each of these last 2 rats when they were sacrificed at 9 months of age.

The hypophysectomized rats for the most part did not thrive as well as the others, presumably because removal of the fur was more difficult for them to endure. All but 1 died when between 75 and 135 days of age, having started but never completed the 3rd hair growth cycle. The 1 surviving hypophysectomized rat (the male) made a good adjustment and was in excellent health when sacrificed for autopsy at 337 days of age. (No remnants of hypophyseal tissue were found.) In this rat the hair cycles started, as in the thryoidectomized rats, at a spot on the back of the neck, which then spread out. Occasionally a second spot appeared somewhat later in the mid-dorsal region, and growth from it likewise proceeded outward. This rat's 6th hair cycle is illustrated in Fig. 1C.

The differences noted between the hair cycles of the operated and control rats were thus of 2 types: changes in the speed with which normally progressing cycles succeeded each other and reached completion, and more fundamental changes in the replacement patterns themselves. The former occurred to a greater or less degree after all the operations, while the latter were found only in thyroidectomized and hypophysectomized rats.

Previous workers have demonstrated alterations in the rate of hair growth which are related to endocrine factors. For instance, Chang (1926) found that thyroidectomy retards hair growth, Snow and Whitehead (1935) observed that hypophysectomized rats take longer to grow a full coat of hair, and Butcher (1937) showed that adrenalectomy stimulates hair growth. We have, however, found no published report which mentions the fundamentally different patterns occurring after removal of thyroid or hypophysis.

SUMMARY

Thyroidectomized or hypophysectomized rats not only showed delay in starting and completing hair-growth cycles, but also had

cycles which proceeded from a different starting point in a direction contrary to the normal. Adrenalectomized rats had greatly accelerated cycles which did not become fragmentary but retained their coherence with advancing age. Gonadectomy had no marked effect on the rats' hair cycles.

REFERENCES

BUTCHER, E. O.: *Am. J. Physiol.* 120: 427. 1937.
CHANG, H. C.: *Am. J. Physiol.* 77: 562. 1926.
DIEKE, S. H.: *Endocrinology* 40: 123. 1947.
SNOW, J. S., AND R. W. WHITEHEAD: *Endocrinology* 19: 88. 1935

URETHANE-INDUCED LYMPHOPENIA IN NORMAL AND ADRENALECTOMIZED RATS

A. DURY AND E. D. ROBIN

From the Department of Physiology, George Washington University, School of Medicine

WASHINGTON, D.C.

A DECREASE in the circulating lymphocytes as well as other physiological changes in normal rats after urethane injections was reported by Hawkins and Murphy (1925). The palliative treatment of human and experimental lymphatic and myelogenous leukemia with urethane has been reported by several investigators (Patterson, Thomas, Haddow and Watkinson, 1946; Haddow and Saxton, 1946; Webster, 1947; Kirschbaum and Lu, 1947; Engstrom, Kirschbaum and Mixer, 1947). Murphy and Strum (1946) reported that urethane treatment of rats with transplanted lymphatic leukemia resulted in a decrease in the white blood cell count and the lymphocytes. Since these investigators also observed adrenal gland hypertrophy, they suggested that this might be of significance in causing the depression of the lymphocytes in their leukemic rats. In this laboratory we had observed enlarged adrenal glands in normal rats after urethanization during the course of an investigation of some of the physiological effects of urethane (unpublished data). Since a lymphopenia can be induced in normal rats with certain of the adrenal cortex steroids (Dougherty and White, 1944), the occurrence of hypertrophied adrenals in urethanized leukemic and normal rats was suggestive of a mode of action of urethane on white blood cells via the adrenal mechanism. The experiments reported here were designed to test this hypothesis. It is the object of this paper to show that the adrenal has no role in the leucopenia and lymphopenia which follows urethanization of the normal rat.

MATERIALS AND METHODS

Adult male normal rats of the Sprague-Dawley strain were used in these studies. The rats were fed Purina Chow and tap water ad. lib. In the case of adrenalectomized rats the tap water was replaced by 1% NaCl solution. The conditions of the experimental procedures were kept as constant as possible throughout the experiments.

Total and differential leucocyte determinations were done by standard procedures on blood obtained from the tails of the rats. The differential leucocyte values were computed after counting at least one hundred white cells on blood smears stained with Wright's blood stain. A 10% urethane solution was used in all the experiments. All urethane injections were given

Received for publication February 26, 1948.

intraperitoneally at a dose level of 750 mg. per Kg. of body weight on Monday, Wednesday, and Friday. Blood samples for leucocyte counts were obtained from the rats at weekly intervals while they were still under the narcotizing effects of a urethane injection. Evipal (10.0 mg. per 100 gm. of body weight) was given intraperitoneally as an anesthetic when blood samples were required from non-urethanized rats or when urethane for anesthetic use was contra-indicated by the experimental procedures. Information regarding other substances administered in the course of these experiments will be described in the section containing the data.

RESULTS

The effect of urethane in normal intact and adrenalectomized rats on total circulating leucocytes is shown in Table 1. The average white

TABLE 1. ALTERATION OF TOTAL CIRCULATING WHITE BLOOD CELLS AFTER URETHANE IN NORMAL RATS

Animal used	WBC per c.mm.	Per cent change
Normal Intact Rats:		
Controls (60)*	21,671 ± 934†	−28.4
Urethane (21)	15,512 ± 1,630	
	P = <0.01	
Adrenalectomized:		
Controls (17)	22,370 ± 1,889	−29.6
Urethane (7)	15,743 ± 1,181	
	P = <0.01	

* Number in ( ) is the number of rats in the experiment.
† S.E. of the Mean.
10% urethane given I.P. tri-weekly at a dose level of 750 mg. per kg. for a total of eight injections.

blood cell value of 60 untreated normal rats is compared with the average value obtained from 21 of these rats after a course of eight intraperitoneal injections of a 10% urethane solution. The average white blood cell value of seventeen untreated adrenalectomized rats is compared with the average value obtained from seven adrenalectomized rats treated with urethane in a similar manner as the intact rats. Urethane treatment of the adrenalectomized rats was started two weeks after the operation. Only those adrenalectomized rats which were found free of adrenal bodies at autopsy were considered in these data. Inspection of Table 1 shows that the average circulating leucocyte value was approximately the same for the urethanized intact and adrenalectomized rats; and that this average value represents an approximate 30% depression from the respective control average figure. It is evident from these data that urethane treatment of normal rats resulted in a leucopenia which was not mediated through the adrenal mechanism.

Evidence of the changed leucocyte composition of the blood of the normal rat after urethane is shown in Table 2. The average values of

the total white blood cell and the absolute lymphocyte and polymorphonuclear leucocytes per c.mm. of blood are given in this table for seventeen intact and eight adrenalectomized rats before and after urethanization. The averages for the control adrenalectomized rats were computed from blood samples taken two weeks after the operation. The urethane treatment was the same for both groups—eight intraperitoneal injections of a 10% solution given tri-weekly at a dose level of 750 mg. per Kg. of body weight. It is evident from these data that the leucopenia which followed the prescribed course of urethanization was the result of an absolute lymphopenia which was of similar magnitude in the norml and the adrenalectomized rats. The absolute number of polymorphonuclear leucocytes was unaffected in these urethanized rats.

TABLE 2. THE LYMPHOPENIC EFFECT OF URETHANE IN NORMAL RATS

Animal used	Lymphocytes absolute number per c.mm.	Polymorphonuclear absolute number per c.mm.	WBC per c.mm.
Normal Intact Rats:			
Controls (17)*	18,824 ±2,512†	4,469 ± 875	23,371
Urethane (17)	9,407 ± 106	5,708 ±1,340	15,524
	P = <0.01	P =0.5	
Adrenalectomized:			
Controls (8)	14,989 ±1,173	6,379 ± 530	21,388
Urethane (8)	10,396 ± 765	4,042 ± 800	14,519
	P = <0.01	P = <0.05	

* Number in ( ) is the number of rats in the experiment.
† S.E. of the Mean.
10% urethane given I.P. tri-weekly at a dose level of 750 mg. per kg. for a total of eight injections.

The change in the composition of the circulating white blood cells after urethane is essentially different from that following the secretion of certain adrenal cortex hormones (Dougherty and White, 1944). These investigators have shown that the lymphopenia which results from adrenal stimulation is at a maximum approximately six hours after the injection of anterior pituitary adrenocorticotrophic hormone and then gradually rises to normal value by the twentieth hour. The lymphopenia which follows urethanization does not appear in the procedure which we followed until after the fifth or sixth injection of urethane. The absolute value of the lymphopenia after urethanization then remains with further injections at approximately 9000 lymphocytes per c.mm. Dougherty and White had also shown that a polymorphonulear leucocytosis was induced shortly after adrenal stimulation with A.C.T.H. and returned to normal absolute value by the ninth hour after injection. Since urethanization did not apparently affect the polymorphonuclear leucocyte picture in the blood of these rats, it was of interest to see whether this would still be so after ad-

renal cortical stimulation of previously urethanized rats.

An inspection of Table 3 shows that the average absolute number of polymorphonuclear leucocytes can be significantly increased in urethanized rats by adrenal cortical stimulation. The average white blood cell and absolute number of lymphocytes and polymorphonuclear leucocytes per c.mm. of blood were determined for seven normal intact rats and eight urethanized rats before and after a series of consecutive injections of an adrenaline solution. The adrenaline was injected according to a procedure recommended by Long and Fry (1945) to attain adrenal cortex stimulation. The adrenaline used was a 0.02%

TABLE 3. THE NEUTROPHILIA-INCITING EFFECT OF ADRENALINE IN URETHANIZED NORMAL RATS

Animal used	Lymphocytes absolute member per c.mm.	Polymorphonuclear leucocytes absolute number per c.mm.	WBC per c.mm.
Normal Intact Rats:			
Controls (7)*	13,125 ±1,084†	2,888 ± 601	16,429
Adrenaline (7)‡	15,930 ±1,412	3,526 ±30	19,714
	P = <0.2	P = 0.3	
Normal Intact Rats:			
Urethane (8)	8,202 ± 446	2,713 ± 376	11,225
Adrenaline (8)	8,644 ± 743	9,456 ±2,488	18,788
	P = <0.5	P = 0.02	

* Number in ( ) is the number of rats in the experiment.

† S.E. of the Mean.

‡ Adrenaline given sub-Q at a dose level of 0.02 mg. per 100 gm. of body weight for four successive hourly intervals.

10% urethane given I.P. tri-weekly at a dose level of 750 mg. per kg. for a total of eight injections.

solution and was injected subcutaneously at four successive hourly intervals at a dose level of 0.02 mg. per 100 gm. of body weight. The adrenaline was administered to the urethane treated rats on the day after the eighth urethane injection. The data of Table 3 show that the intact non-urethanized rats were unaffected by the adrenaline injections insofar as their white blood cell composition was concerned. However, the urethanized rats had a completely distorted leucotyte picture twenty hours after the adrenaline injections. These rats had a polymorphonuclear leucocytosis along with a previously existing urethane-induced lymphopenia. It is apparent that urethane's effect on the white blood cells is not restricted to the lymphocytes; and that urethane is somehow related to the adrenal cortex mechanism as shown by its effect on the polymorphonuclear leucocytes.

DISCUSSION

Three general modes of urethane action are extant in the literature to explain its physiological effects on blood cells and tissues in general. Webster (1947) suggests that urethane induces a factor to which leukemic cells are especially sensitive. Kirschbaum and Lu (1947) em-

phasized their observation that there was an increased number of mature circulating white blood cells after urethane treatment of leukemic animals and suggested that these cells were more readily discarded by the leukemic animal. The second possible mode of action of urethane was that inferred from the hypothesis of Murphy and Strum (1946) that the hypertrophied adrenals secreted an increased quantity of a lymphocytolytic factor. This would place urethane in the large category of substances whose general effects upon the organism is explained by the General Adaptation Syndrome (Selye, 1946). Since the adrenal cortex is fundamental in inducing those changes from the physiological norm which are seen in the Adaptation Syndrome, the data presented in Tables 1 and 2 on the adrenalectomized rats makes this explanation untenable. The enlarged adrenal glands recorded by Murphy and Strum and by the authors in leukemic rats and normal rats, respectively, might be an alarm reaction to urethane secondarily but directly the result of the liberation of proteolytic products by this compound (Selye, 1946). This third possible mode of action of urethane is supported by the observations of several investigators—Warburg (1910) had shown that phenyl urethane in small amounts arrested mitosis and cell division in fertilized eggs of the sea urchin; Guyer and Claus (1947) reported the practical abolition of mitosis in the cornea of the rat; Engstrom, Kirschbaum and Mixer (1947) reported a decrease in the size of the spleen and lymph nodes of urethanized animals; and Kirschbaum and Lu (1947) observed a decrease in the number of mitotic figures in marrow myeloid cells of mice with myelogenous leukemia after urethane injections. Since we have shown that a lymphopenia can be induced with urethane in normal adrenalectomized rats and recently Lu and Kirschbaum (1947) reported that the adrenal mechanism was not involved in the effects of urethane in leukemic mice, it appears that urethane behaves as a mitotic poison insofar as it affects the lymphocytes. The alteration of the polymorphonuclear leucocyte picture after adrenal stimulation of urethanized normal rats cannot be explained, but it is under investigation.

SUMMARY

A leucopenia and an absolute lymphopenia of similar magnitude was induced in normal intact and normal adrenalectomized rats following the intraperitoneal administration of a 10% urethane solution. The evidence presented here showed that the adrenal glands were not involved in these changes in the blood picture. A possible mode of urethane action was discussed.

A relation of urethane with the adrenal mechanism was suggested by a polymorpho-leucocytosis which was induced in urethanized rats twenty hours after adrenal cortical stimulation.

REFERENCES

ENGSTROM, R. M., A. KIRSCHBAUM AND H. W. MIXER: *Science* 105: 255. 1947.
DOUGHERTY, T. F., AND A. WHITE: *Endocrinology* 35: 1. 1944.
GUYER, M. F., AND P. E. CLAUS: *Proc. Soc. Exper. Biol. & Med.* 64: 3. 1947.
HADDOW, A., AND W. A. SAXTON: *Nature, London* 157: 500. 1946.
HAWKINS, J. A., AND J. B. MURPHY: *J. Exper. Med.* 42: 609. 1925.
KIRSCHBAUM, A., AND C. S. LU: *Proc. Soc. Exper. Biol. & Med.* 65: 62. 1947.
LONG, C. N. H., AND E. G. FRY: *Proc. Soc. Exper. Biol. & Med.* 59: 67. 1945.
LU, C. S., AND A. KIRSCHBAUM: Fourth International Cancer Research Congress, St. Louis, Mo., Sept. 2–7, p. 105. 1947.
MURPHY, J. B., AND E. STRUM: *Science* 104: 427. 1946.
PATTERSON, E., Ap. I. THOMAS, A. HADDOW AND J. M. WATKINSON: *Lancet* 1: 677. 1946.
SELYE, H.: *J. Clin. Endocrinol.* 6: 117. 1946.
WARBURG, O.: *Ztschr. f. Physiol. Chem.* 66: 305. 1910.
WEBSTER, J. J.: *J.A.M.A.* 135: 901. 1947.

NOTES AND COMMENTS

GOITEROUS CHICKS FROM THYROPROTEIN-FED HENS

It has been shown that goiterous dams, or dams fed goiterogenic diets produce offspring with enlarged thyroids (Kalkus, 1920; Gassner and Wilgus, 1940; Hughes, 1944; Goldsmith, Gordon and Charriper, 1945; Andrews and Schnetzler, 1945; Hollander and Riddle, 1946). Goiters in these studies resulted from iodine deficiency *per se*, thiourea, and thiouracil. In mammals the goiterogenic agents are transmitted to the young through the placenta and the milk (Hughes, 1944; Goldsmith, Gordon, and Charriper, 1945). In birds the agents are transmitted through the egg (Andrews and Schnetzler, 1945).

There is a lack of information on the effect of hyperthyroidism of the dam on the thyroid size of her progeny. In the present paper thyroid weights of newly hatched chicks from normal and thyroprotein-fed hens are compared.

METHODS

In January and February 1948, three hatches were made from two groups of 10-month old Rhode Island Red hens. One group of 80 hens had been fed 0.02% thyroprotein[1] in the ration continuously from day 1. A control group of 80 comparable hens had been fed the same basal ration. The previous history of these birds has been described in detail elsewhere (Wheeler, Hoffmann, and Graham, 1948; Wheeler and Hoffmann, 1948). The chicks were reared in electric brooders with raised screen floors and were fed a conventional all-mash starting ration. On day 2, 8, and 16 a sample of chicks was sacrificed, the thyroid glands removed and weighed to the nearest 0.1 mg. on a Roller-Smith balance.

RESULTS

The thyroid gland weights of the 2-day old male and female chicks from the three hatches are presented in Table 1. In every case the thyroid glands of the chicks from thyroprotein-fed hens are significantly heavier. A sex difference in response to the treatment is seen in the significantly greater thyroid enlargement in the female chicks. Figure 1 presents a composite curve of the change in thyroid weight from day 2 to day 16. Thyroid weight in the control chicks decreased somewhat during the first week and increased rather sharply during the second week after hatching. Thyroid weight in the experimental chicks decreased sharply during the first week and increased somewhat the second week. By the 16th day weights were comparable in both groups.

There were no observed differences in behavior, or in rate of bodily growth between the two groups of chicks.

Received for publication February 27, 1948.

[1] Thyroprotein (Protamone) was generously supplied by Dr. W. R. Graham, Jr., Cerophyl Laboratories, Kansas City, Mo. This substance, iodinated casein, contains 3.0% thyroxine according to the manufacturer's chemical assay.

TABLE 1. THYROID WEIGHTS OF 2-DAY OLD CHICKS FROM THYROPROTEIN-FED HENS

Diet of hen[1]	No. of chicks	Sex	Av. thyroid wt. (mg.)	t value[2]	Date of hatch
Treated	5	M	9.60	5.93	
Control	5	M	4.96		1-1-48
Treated	5	F	10.08	4.09	
Control	5	F	4.68		
Treated	7	M	7.00	2.32	
Control	5	M	4.22		1-13-48
Treated	5	F	8.36	5.28	
Control	5	F	3.84		
Treated	5	M	6.30	3.56	
Control	9	M	4.00		2-5-48
Treated	5	F	8.70	4.03	
Control	5	F	3.90		
All treated	17	M	7.63	6.07	
All controls	19	M	4.39		
All treated	15	F	9.05[3]	7.35	
All controls	15	F	4.14		

[1] Diet of treated hens contained thyroprotein at 0.02% level.
[2] Calculated for the mean thyroid weight of each treated group and the corresponding control group.
[3] Thyroid weight of treated females significantly greater than that of treated males ($t=2.1$).

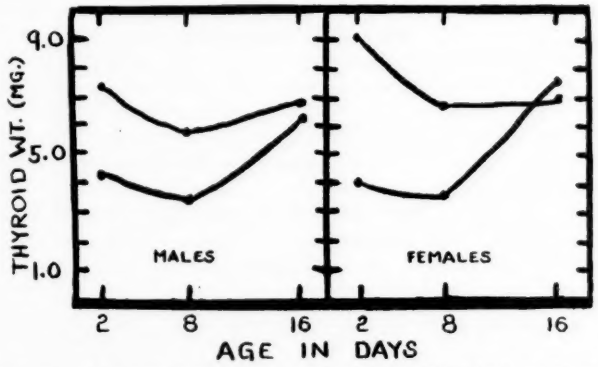


FIG. 1. Composite curve showing change in weight of thyroid from day 2 to day 16. The lower line in each case represents the control chicks, and the upper line the chicks from thyroprotein-fed hens.

COMMENT

The effect on the chick thyroid of feeding thyroprotein to the hen is the antithesis of the effect of feeding this substance directly to the chick or to the adult. Feeding thyroprotein directly results in marked thyroid involution (Wheeler, Hoffmann, and Graham, 1948; Wheeler and Hoffmann, 1948).

The results obtained here bear remarkable similarity to those occurring

when thiourea or thiouracil are administered to pregnant rats or to hens (Goldsmith, Gordon, and Charriper, 1945; Andrews and Schnetzler, 1945). With each of these treatments (thyroprotein, thiourea, thiouracil) the offspring have enlarged thyroids. Further similarity is seen in the return to normal thyroid weight when the chicks are fed a conventional ration. Goldsmith and coworkers reported that the enlarged thyroids of newborn rats from thiourea-fed mothers became reduced soon after the baby rats were fed a standard diet. The sex difference in response (greater thyroid enlargement in female chicks) reported here is similar to the observations of Glazener and Jull (1946) in thiouracil-fed chicks.

The goiterous chicks of thyroprotein-fed hens differ from the goiterous offspring of iodine-deficient dams. The latter progeny show marked debility and weakness (Kalkus, 1920; Wilgus and Gassner, 1941; Hollander and Riddle, 1946). The experimental chicks in our study were indistinguishable from normal chicks in vigor, behavior, and rate of growth.

We are unable to explain adequately the difference in post-hatching rate of growth of the normal chick thyroid shown in our data and the data of Albert, *et al.* (1947). The latter workers showed a regular increment in thyroid weight from day 1 to day 25, as contrasted to the initial decline at 8 days which we observed. Two factors might account for this difference: different breeds were studied, and the weights were taken at different seasons. Albert and coworkers obtained their data from White Leghorn males in May, June, and July, while our weights were from Rhode Island Red chicks in January and February.

These findings are admittedly preliminary and work is in progress to further study the mechanism involved in the production of goiterous chicks from hyperthyroid dams.

SUMMARY

Chicks from hens fed 0.02% thyroprotein (containing 3.0% thyroxin by weight) have significantly enlarged thyroids at the time of hatch. When these chicks are fed a standard ration their thyroids become reduced within the normal range by the 16th day following hatch. These results are of interest since thyroprotein fed directly to chicks or to adults produces marked involution of the thyroid.

ROBERT S. WHEELER AND EDMUND HOFFMANN
Department of Poultry Husbandry
The University of Georgia, Athens, Georgia

REFERENCES

ALBERT, A., R. W. RAWSON, C. RIDDELL, P. MERRILL AND B. LENNON: *Endocrinolgy* 40: 361. 1947.
ANDREWS, F. N., AND E. E. SCHNETZLER: *Endocrinology* 37: 382. 1945.
GASSNER, F. X., AND H. S. WILGUS, JR.: *Poultry Sci.* 19: 349. 1940.
GLAZENER, E. W., AND M. A. JULL: *Poultry Sci.* 25: 533. 1946.
GOLDSMITH, E. D., A. S. GORDON AND H. A. CHARRIPER: *Am. J. Obst. & Gynec.* 49: 197. 1945.
HOLLANDER, W. F., AND O. RIDDLE: *Poultry Sci.* 25: 20. 1946.
HUGHES, A. M.: *Endocrinology* 34: 69. 1944.
KALKUS, J. W.: *Wash. Agr. Exper. Sta. Bul.* 156. 1920.
WHEELER, R. S., E. HOFFMANN AND C. L. GRAHAM: *Poultry Sci.* 27: 103. 1948.
WHEELER, R. S., AND E. HOFFMANN: *Poultry Sci.* 27: (In press). 1948.
WILGUS, H. S., JR., AND F. X. GASSNER: *Proc. Soc. Exper. Biol. & Med.* 46: 290. 1941.

NEW BOOK

GEORGE CRILE, An Autobiography. Edited, with Sidelights, by Grace Crile. In Two Volumes. J. B. Lippincott Company, Philadelphia and New York. 1947.

This is an extraordinary autobiography. From humble origins, with only those educational opportunities which he could earn for himself, George Washington Crile rose to the foremost rank of American and international surgery; he served his country well in two wars, being one of the few medical reserve officers ever honored with the grade of Brigadier General; and together with his lifelong friend, "Ed" Lower, he founded one of the great surgical clinics of the world. These accomplishments were not in themselves primary objectives. They came as the logical result of Dr. Crile's prodigious industry. His primary interest in life was to learn the how and why of things. His mind was keen, tireless, fearless, and passionately inquisitive. When he could not see or find the answer to a problem he dreamed up an answer.

No biographer could have portrayed the nature and direction of Dr. Crile's thinking as well as this collection of personal notes and sketches, scribbled hastily on bits of paper whenever the author had a moment on a train, a street car, or even in the bath tub. The material has been assembled and edited by Mrs. George Crile who has contrived to give a cohesiveness to the story without destroying the basic character of the original notes.

These are not the profound reflections of a great man looking back over a life of memorable accomplishment. These are the thoughts, the hopes, and the works of a man, forever young, constantly looking ahead, driving, seeking, finding, dreaming, and living. The incidents are succinctly but often minutely described and their range is stupendous: a hair raising description of intubating a child with diphtheria in the days when Dr. Crile was a "horse and buggy doctor"; the first total laryngectomy done in America; early experiences in spinal anesthesia; studies in shock and in thyroid disease; experiences in two wars; hunting big game in an extraordinary study of comparative anatomy and physiology; and through it all a constantly recurring glimpse of deep, happy family associations.

There is struggle, disappointment, and, as in the great Cleveland Clinic fire, disaster, but there is little sadness and no bitterness in these pages. The loneliness and frustration which so obviously gripped Harvey Cushing in his last years were completely unknown to Crile. He never accepted defeat. He overcame it or rationalized it out of his ken. He mastered his impending blindness by developing an operation for hypertension which could be done by touch alone. At 75, practically blind, harassed by personal financial difficulties, his son, Barney, departing for war, and his Clinic facing possible disintegration, he had the amazing intellectual vigor to take an active and instrumental part in developing a pressurized flying suit for the navy.

The editorial sidelights by Mrs. Crile contain some of the best writing in the book and give an insight to Dr. Crile's life which would be missed without them.

On the debit side there is little of lasting literary or historical value. The presentations of his theory of shock, of the "thyroid-adrenal-sympathetic complex" and of the nature of life and cellular activity are too long for the lay reader and too short for those who might be interested. Crile saw and contributed to the great rise of American surgery. He was an intimate of all the great surgeons of his day and his sharp analytical mind might well have left a valuable record of the men and their works. But his comments about his contemporaries are for the most part casual and superficial. His struggle with Allen to gain control of the Lakeside Hospital, his difficulties with the Army in 1918, the bitterness and animosity evoked by his efforts to put medical theory into practice—all are mentioned or implied but the "inside story" is lacking.

Moreover, there is no critical analysis of Crile's own contributions. His conviction that the liver was a vital organ in the cause of shock, his studies of methods of resuscitation, his early attack upon the sympathetic nervous system in the treatment of hypertension are examples of his work which although revolutionary have proved to be sound and far in advance of his time. Others, such as "exhaustion of the vasomotor center in shock" and denervation of the adrenal glands for hyperthyroidism have not been substantiated. The unitiated reader will be lost in a welter of ideas for this book fails to distinguish the wheat from the chaff.

However, these are minor criticisms. The man was indomitable and the reader who follows his life in these pages receives a richly rewarding lesson in how to live and to work.

J. Englebert Dunphy

ASSOCIATION NOTICE

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

PALMER HOUSE, CHICAGO

PROGRAM

FRIDAY, JUNE 18, 1948

8:30 A.M. Registration

I. 9:30 A.M. Red Lacquer Room

J. S. L. BROWNE, *presiding*

1. PSEUDO-GLANDULAR DISTURBANCES.
   by Hugo R. Rony
2. SYNDROME OF CRYPTORCHIDISM, HEART DISEASE AND DERMATOSIS.
   by S. J. Glass
3. CONSTITUTIONAL PRECOCIOUS PUBERTY CONTROLLED BY ANDROGEN THERAPY.
   by S. Charles Freed and Minnie Goldberg
4. A STUDY OF THE BIOLOGICAL ACTIVITY AND THE MAGNITUDE OF ENDOGENOUS ANDROGEN PRODUCTION IN A CASE OF ADRENOGENITAL SYNDROME.
   by Anne C. Carter and Ephraim Shorr
5. PSEUDOHERMAPHRODISM. EARLY AND LATE RECOGNITION.
   by M. James Whitelaw
6. CLINICAL, LABORATORY, OPERATIVE AND POSTMORTEM OBSERVATIONS IN INFANTS AND CHILDREN WITH MULTIPLE CONGENITAL MALFORMATIONS (TURNER'S SYNDROME, OVARIAN AGENESIS AND RELATED COMBINATIONS).
   by Frank L. Plachte (introduced by Henry H. Turner)
7. A SYNDROME CHARACTERIZED BY HYPERCALCEMIA, CALCINOSIS, AND RENAL INSUFFICIENCY FOLLOWING PROLONGED INTAKE OF CALCIUM AND ALKALI.
   by Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright and John E. Howard
8. HYPOPARATHYROIDISM, WITH MENTAL TROUBLES AND ECTODERMAL DISORDERS.
   by Manuel Villaverde
9. TREATMENT OF FAR ADVANCED INOPERABLE CARCINOMA OF THE BREAST WITH ESTROGENS AND ANDROGENS.
   by Samuel G. Taylor, III, Danely Slaughter (by invitation) and Frederick W. Preston (by invitation)
10. HORMONAL FACTORS INVOLVED IN THE REGULATION OF BODY TEMPERATURE DURING MENSTRUAL CYCLE AND PREGNANCY.
    by Charles L. Buxton and William B. Atkinson
11. THE EFFECTS OF CERTAIN STEROIDS—INTRAMUSCULAR AND SUBLINGUAL —ON THE BASAL BODY TEMPERATURE OF THE ADULT HUMAN MALE.
    by Robert M. Perlman

II. 2:00 P.M. Red Lacquer Room

C. N. H. LONG, *presiding*

12. A SIMPLIFIED HYPOPHYSECTOMIZED RAT ADRENAL ASCORBIC ACID BIOASSAY METHOD FOR ADRENOCORTICOTROPHIN (A.C.T.H.); SPECIFICITY AND APPLICATION TO PREPARATIVE PROBLEMS.
by Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch

13. CONTENT OF ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) IN THE RAT PITUITARY UNDER OPTIMAL AND STRESSFUL ENVIRONMENTAL CONDITIONS.
by George Sayers, Marshal Merkin (by invitation) and J. N. Tortoreto (by invitation)

14. THE ACTIVATION OF THE ADRENAL CORTEX BY INSULIN HYPOGLYCEMIA.
by H. Gershberg (by invitation) and C. N. H. Long

15. INFLUENCE OF ADRENOTROPHIC HORMONE ON SODIUM EXCRETION IN HYPOPHYSECTOMIZED RATS.
by Betty L. Rubin (by invitation) and Ralph I. Dorfman

16. FACTORS INFLUENCING THE CORTICOTROPHIN PRODUCTION OF THE ANTERIOR PITUITARY.
by Hans Selye

17. THE USE OF ADRENOCORTICOTROPHIN AS A TEST OF ADRENAL CORTICAL RESERVE.
by George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation) and A. Gorman Hills (by invitation)

18. OBSERVATIONS ON THE PITUITARY-ADRENAL RESPONSE FOLLOWING EPINEPHRINE INFUSION IN MAN.
by Lillian Recant (by invitation), Peter H. Forsham (by invitation) and George W. Thorn

19. FATE AND METABOLIC ACTION OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.).
by Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels

20. METABOLIC CHANGES FOLLOWING THE ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) TO NORMAL HUMANS.
by H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne

21. THE EFFECT OF ADRENOCORTICOTROPHIN ON ANTIBODY LEVELS IN NORMAL HUMAN SUBJECTS.
by P. H. Herbert and J. A. de Vries (introduced by J. S. L. Browne)

22. A COMPARISON OF THE EFFECT ON BONE FORMATION OF THE HYPERADRENOCORTICISM OF CUSHING'S SYNDROME WITH THAT INDUCED BY ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.).
by Frederic C. Bartter (by invitation), Anne P. Forbes and Fuller Albright

23. ADRENAL CORTICAL UNRESPONSIVENESS IN PATIENTS WITH GASTRIC CANCER.
by Edward C. Reifenstein, Jr., N. F. Young (by invitation), Aurelia Potor (by invitation), Benedict Duffy (by invitation) and F. Homburger (by invitation)

24. THE EXCRETION OF ADRENAL METABOLITES IN HUMAN URINE.
by Konrad Dobriner, Seymour Lieberman (by invitation) and C. P. Rhoads (by invitation)

III. Annual Dinner

7:30 p.m.—Red Lacquer Room, Palmer House
Presentation of E. R. Squibb and Sons Award for 1948.
Presentation of Ciba Award for 1948.
Presentation of Ayerst, McKenna and Harrison Fellowship for 1948.
Warren O. Nelson, Chairman of the Committee on Awards 1947–48.

President's Address: C. N. H. Long, Yale University

Saturday, June 19, 1948

IV. 9:00 a.m. Red Lacquer Room

R. G. Hoskins, *presiding*

25. Preparation of Crystalline Growth Hormone.
by Jacob B. Fishman (by invitation), Alfred E. Wilhelmi (by invitation) and Jane A. Russell

26. The Influence of Purified Growth Hormone on Fasting Metabolism.
by Clara M. Szego and Abraham White

27. Unpredictable Effects of Growth Hormone Preparations on Nitrogen Storage.
by Paul Bartlett (by invitation) and Oliver H. Gaebler

28. Studies in Growth. I. The Effects of Androgen in Gigantism and Acromegaly.
by Lawrence W. Kinsell, George D. Michaels (by invitation), Choh Hao Li (by invitation) and William E. Larsen (by invitation)

29. The Effect of Iodine Injections on Energy Metabolism and Plasma Protein-bound Iodine of Rats.
by S. B. Barker and H. J. Lipner (by invitation)

30. The Effect of Pituitary and Non-pituitary Gland Factors on the Formation of Intracellular Colloid Droplets in the Thyroid Gland in Vivo and in Vitro.
by Samuel Dvoskin

31. Inactivation of the Exophthalmic, Thyrotropic and Ketogenic Principles of Anterior Pituitary Extract by Iodination.
by William McK. Jefferies

32. Newer Methods of Antagonizing Hyperthyroidism.
by Robert H. Williams, Rene F. Tagnon (by invitation), Herbert Jaffe (by invitation), Beverly T. Towery (by invitation) and Walter F. Rogers (by invitation)

33. The Use of Radioactive Iodine (I131) in the Study of Normal and Disordered Thyroid Function in Man.
by Sidney C. Werner and Edith Quimby (by invitation)

34. The Effect of Thyroid Stimulating Hormone on the Function of Human Normal and Malignant Thyroid Tissue.
by J. B. Trunnell (by invitation), R. W. Rawson, L. D. Marinelli (by invitation) and Ruth Hill (by invitation)

35. The Relation Between Infant Birthweight and Subsequent Development of Maternal Diabetes Mellitus.
by Joseph P. Kriss and Palmer H. Futcher (introduced by Cyril M. MacBryde)

V. 2:00 p.m. Red Lacquer Room

A. T. Kenyon, *presiding*

36. Absorption and Excretion of Chorionic Gonadotrophin When Administered Intramuscularly to Women.
by J. T. Bradbury and Willis E. Brown

37. The Renal Clearance of Chorionic Gonadotrophic Hormone in Pregnancy and in Neoplasm of the Testis.
by C. F. Gastineau (by invitation), A. Albert and L. M. Randall (by invitation)
38. The Metabolic Response to Chorionic Gonadotrophin in Young Men.
by Kathryn Knowlton (by invitation) and Allan T. Kenyon
39. Blood Gonadotrophin Studies During Pregnancy in Relation to the Fetal Sex.
by H. E. Niegurgs and Robert B. Greenblatt
40. On the Principal Estrogenic Constituents of the Urine of the Stallion.
by Louis Levin
41. Mechanism of Inactivation of α-Estradiol by Rat Liver in Vitro.
by R. H. deMeio (by invitation), A. E. Rakoff, A. Cantarow and K. E. Paschkis
42. Cozymase in the Hepatic Inactivation of α-Estradiol.
by Richard L. Coppedge (by invitation), Albert Segaloff, Herbert Sarett (by invitation) and Aaron Altshul (by invitation)
43. Interference with Estrogen-Induced Growth in the Female Genital Tract by Folic Acid.
by Roy Hertz
44. The Relation of Folic Acid to the Action of Estrogens.
by Irene T. Kline (by invitation) and Ralph I. Dorfman
45. Fluorescent Phenomena of the Vulva Associated with Sex Hormone Metabolism.
by M. Sydney Margolese
46. Testicular Deficiency: A Clinical and Pathological Study.
by R. Palmer Howard, Ronald C. Sniffen (by invitation) and Fred A. Simmons
47. A Comparison of the Effect of Various Androgens on the Temporal Muscle and Organs of the Castrated Male Guinea Pig.
by Charles D. Kochakian and Jane Harrison Humm (by invitation)

VI. Annual Business Meeting

5:00 p.m. Red Lacquer Room

Papers Read by Title

48. The Use of Whole Adrenal Cortical Extract in Experimental Infections.
by Erwin P. Vollmer, James D. Gillmore (by invitation), Leo Cravitz (by invitation) and J. E. Samsell (by invitation).
49. The Work Performance of Adrenalectomized Rats Given Continuous Intravenous Infusions of Glucose.
by Dwight J. Ingle and James E. Nezamis (by invitation)
50. Sublinqual Administration of Desoxycorticosterone Acetate in the Treatment of Addison's Disease.
by Evelyn Anderson, Lawrence W. Kinsell, Troy C. Daniels (by invitation) and Edward Henderson
51. Excretion of Adrenal Metabolites Following the Administration of Adrenocorticotrophic Hormone to Normal Human Subjects.
by Eleanor H. Venning, V. E. Kazmin (by invitation), Miriam Ripstein (by invitation), H. T. McAlpine (by invitation) and M. M. Hoffman
52. The Effect of 11-Dehydrocorticosterone on Fecal Fat Excretion.
by Grace E. Bergner (by invitation), Roger A. Lewis (by invitation),

Frances W. Stout (by invitation), George W. Thorn and Kendall Emerson, Jr.

53. An Estimation of the Quantity of 11-17-Oxysteroid Excretion by the Human Adrenal Stimulated by A.C.T.H.
by A. Gorman Hills (by invitation) and George W. Thorn
54. Isolation of Urinary Steroids from a Patient with Apparent Adrenal Involvement.
by A. M. Miller (by invitation) and Ralph I. Dorfman
55. The Effect of Adrenalectomy and Desoxycorticosterone Acetate Administration upon the Ecg Response of the Rat to Cardiac Glycosides.
by Herbert S. Kupperman, Joseph G. Benton (by invitation) and Arthur C. DeGraff (by invitation)
56. Activation of the Adrenal Cortex in Human Subjects Following Electroconvulsive Therapy (E.C.T.) and Psychomotor Stress.
by R. A. Cleghorn and A. J. Goodman (by invitation), B. F. Graham, M. H. Jones and N. K. Rublee
57. Effect of Adrenal Cortical Compounds on Electrolyte Metabolism of a Patient with Addison's Disease During High Sodium Chloride Intake.
by Aurelia Potor (by invitation), Nelson F. Young (by invitation), F. Homburger (by invitation) and Edward C. Reifenstein, Jr.
58. Nitrogensparing (Protein-Anabolic) Action of Thyroid Hormone.
by J. Rupp (by invitation) and K. E. Paschkis
59. Thiouracil Effect on Plasma and Liver Protein Concentrations.
by James H. Leathem
60. Radioiodine Uptake by the Thyroid as an Aid in Differential Diagnosis.
by S. M. Seidlin, E. Oshry (by invitation), I. Rossman (by invitation) and L. Leiter (by invitation)
61. Thyroid Uptake of Radioactive Iodine in the Normal and Hypometabolic Human.
by Martin Perlmutter (by invitation) and Peter H. Forsham (by invitation)
62. Cellular Involution in the Thyroid.
by Nathan B. Friedman
63. Methyl Thiouracil in the Treatment of Thyrotoxicosis.
by Grosvenor W. Bissell, John M. Benny (by invitation), Victor Totah (by invitation) and Florence Gilbert (by invitation)
64. Modification of the Estrual Cycle of the Ewe by the Use of Progesterone; The Effect upon Subsequent Ovulation Rate and Fertility of Ova.
by R. H. Dutt (by invitation) and L. E. Casida
65. Effects of Various Estrogenic Preparations on the Vaginal Mucosa.
by Mildred Vogel (by invitation), Thomas H. McGavack and Joseph Mellow (by invitation)
66. The Use of the Vaginal Smear in the Assay of Estrogens Given Orally or Intramuscularly.
by Willis E. Brown and J. T. Bradbury
67. The Similarity of Estrogenic Effect in Premenstrual Tension, Menstrual Anomalies, Chronic Cystic Mastitis and Cancer of the Breast.
by Joseph H. Morton
68. Hyperestrogenism Treated with Lactogenic Hormone (Prolactin).
by Manuel Villaverde

69. The Factor of Rhythm in Experimental Menstruation.
by Doris H. Phelps
70. Hormonal Pellets in the Management of the Menopausal Syndrome.
by Robert B. Greenblatt and Roland R. Suran (by invitation)
71. The Effect of Hypophysectomy on the Ovulability of the Ovarian Follicle of the Domestic Hen.
by Irving Rothchild and R. M. Fraps
72. Prognostic Value of Pregnandiol Excretion in Threatened Abortion with Special Reference to the Effects of Diethstilbestrol.
by A. R. Abarbanel
73. Further Studies on the Endometrial Cups of the Pregnant Mare.
by H. H. Cole and G. H. Hart
74. Inactivation of Posterior Pituitary Antidiuretic Hormone of the Liver.
by W. J. Eversole, J. H. Birnie (by invitation) and Robert Gaunt
75. The Estimation of Dehydroisoandrosterone and Related Compounds in Human Urine by a Modification of the Pettenkoffer Reaction.
by Richard L. Landau and Kathleen Lugibihl (by invitation)
76. Clinical Evaluation for 17 Ketosteroids by the Rapid Method.
by T. H. McGavack, S. Kenigsberg, A. M. Shearman and K. J. Drekter
77. The Application of Paper Partition Chromatography to Ketosteroids.
by Robert B. Burton (by invitation), Alejandro Zaffaroni (by invitation) and E. Henry Keutmann
78. A Rapid Modification of the Zimmerman Test for Ketosteroids.
by Sidney Pearson and Sylvester Giaccone (by invitation)
79. A Fluorometric Method for the Determination of Estrone and Estradiol in Human Urines.
by Joseph W. Jailer
80. A Clinical Bio-Assay for Chorionic Gonadotrophin.
by A. Albert
81. Specificity of a Colorimetric Method for Dehydroisoandrosterone in Urine Extracts.
by Paul L. Munson, Mary Ellen Jones (by invitation), Philip J. McCall (by invitation) and T. F. Gallagher (by invitation)
82. The Colorimetric Determination of Sodium and Its Application in the Study of Sodium and Chloride Balance.
by Joseph W. Goldzieher and Gilbert Stone (by invitation).
83. Depression of Lymphocyte Count after Orally Administered Glucose.
by Paul A. Marks (by invitation), Dorothy T. Marks (by invitation) and Joseph W. Jailer
84. Pitfalls in the Diagnosis of Diabetes.
by Bernard A. Watson
85. Electrolyte Balance Studies in the Uncontrolled and Controlled Diabetic State.
by Jonas Weissberg (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Drekter
86. The Role of the Endocrine Glands in Body Temperature Regulation.
by H. E. Nieburgs and Robert B. Greenblatt
87. The Lipogenic Activity of Propythiouracil, Triphenylchloroethylene and Hexestrol in Chickens.
by R. George Jaap

88. The Influence of the Pituitary Gland on Blood Vessel Development. The Effect of Concentrated Pituitary Extract on the Isolated Kidney.
by Robert C. Moehlig and Louis Jaffe (by invitation)
89. Results of Prolonged Medical Treatment of Obesity with Diet Alone, Diet and Thyroid Preparations, and Diet and Amphetamine.
by David Adlersberg and Martin E. Mayer (by invitation)
90. The Urethral Smear in the Normal Human Male.
by Mildred T. Vogel (by invitation), Thomas H. McGavack and Henry Kammandel (by invitation)
91. The Specific Ovarian Hyperemic Inducing Effect of Luteinizing and Luteotrophic Hormones.
by Herbert S. Kupperman, W. H. McShan and Roland K. Meyer